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EDITORIAL

INSECTICIDES AND WEED KILLERS

In 1950 two young men died as the result of handling a chemical crop spraying agent known as DNC. This tragic incident coupled with others less severe prompted the Minister of Agriculture and Fisheries to convene a Working Party whose duty it was to report on Toxic Chemicals in Agriculture. The first Report, published in 1951, dealt with the dangers to man and emphasised the need for suitable protective measures. The second and third were published in 1953 and early this year, and dealt respectively with Residues in Food and Risks to Wild Life.

The third report is of particular interest to the veterinary profession in that it includes details of casualties to farm and domestic stock as well as to wild life, and of some of the measures that can be taken to minimise the risks of exposure.

In taking stock of the present situation at least three factors must be considered. First, more than two and a half million acres of crops are sprayed per annum in the United Kingdom. This is more than five times the acreage that was sprayed in 1950. Secondly, many varieties of chemicals are in current use and they have widely different physiological properties. Thirdly, and probably this is the most important fact of all, the development of toxic chemicals has far outstripped our knowledge of their precise mode of action in the animal body. While fairly adequate facts are available on the minimum quantities necessary to cause death or serious injury to an animal, relatively little information exists on the chronic effects which may follow the continuous absorption

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of minute quantities of these poisons. For example, what are the effects of some of the more commonly used agents on ruminant metabolism, on milk production, on fertility and on general health?

These questions require an answer and call for an investigation to ascertain the cumulative power of the poisons used and the animal's ability to get rid of them and to repair any damage that they may have caused. A single minute dose of poison may cause so little immediate damage that it cannot be identified accurately by existing clinical, pathological or biochemical means, but the cumulative effect of many small doses may cause serious damage, as is the case when DNC and some of the organo-phosphorus compounds are used.

These are the immediate problems but it would be wise to look to the future. One of the great drawbacks of many of the existing herbicides and pesticides is their lack of specificity, for example some substances that kill the red mite will kill also bees, cattle and man. The lethal dose may vary according to the species but the broad effects are similar. Two parallel lines of research are indicated, first, the production of new substances that are more specific in their action towards a limited range of weeds or insects and secondly, the search for substances that are less harmful to the higher animals than those that are at present in use.

The uncontrolled use of non specific insecticides may bring serious troubles in its wake and destroy the beneficient synergism on which all life depends.

No effort should be spared in the attempt to solve these current and future problems.

While there is no doubt that most of these artificial aids to agricultural economy are of considerable immediate value, it must be remembered that their misuse can lead to serious trouble that may be more lasting than the benefits which they confer.

This is not an uncommon property of most of man's inventions.

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GENERAL ARTICLES

HÆMOLYTIC DISEASE OF NEWBORN PIGS CAUSED BY MATERNAL ISO-IMMUNISATION

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HÆMOLYTIC disease of the newborn is well recognised in man and is due to iso-immunisation of the mother by fœtal red cell antigens of the rhesus and other blood groups. Within the last decade the condition has been observed in foals and more recently in pigs, but, so far as we are aware, its existence as a naturally occurring disease† has not been established in any other species, although the possibility of a comparable condition in other animals has been suggested.

The classic pattern of hæmolytic disease consists in the mating of serologically incompatible parents, and in man, the rhesus blood groups play the major part in its production. In such cases the fœtus inherits antigen from the father; this is passed to the mother and against which she produces antibody. Under certain conditions of placental permeability, this antibody may pass back into the fœtus and so bring about its death *in utero*. The antibody may be concentrated in the mother's colostrum and in this case the fœtus is born normally, and shows evidence of disease a short time after suckling, with destruction of its red blood cells by the maternal antibody. This results in anæmia, often of a profound kind, and it may be accompanied by clinical jaundice and death. The condition of *icterus gravis neonatorum* has long been appreciated in human and veterinary medicine, but recently it is known to be a common symptom of hæmolytic disease.

The first cases of iso-immunisation of pregnancy in animals to be reported were those of Caroli and Bessis (1947), who drew attention to jaundice in newborn mules; in later papers they gave a comprehensive description of the serology, hæmatology and pathology of the disease. In the following year Coombs and his colleagues reported on six cases of hæmolytic disease in thoroughbred foals (Coombs *et al.*, 1948). Up to the present time the disease as it occurs in pigs has been reported from Hungary, Great Britain and the United States (Szent and Szabo, 1952; Buxton and Brooksbank, 1953*a*, *b*; Doll and Brown, 1954). Kershaw (1950) described a clinical condition of baby pigs which he recognised as being similar to hæmolytic disease in foals.

The present paper describes in detail the investigations of porcine hæmolytic disease which were briefly reported earlier (Buxton and Brooksbank, 1953*a*, *b*).

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† It is perhaps not correct to talk of this as a naturally occurring disease until iso-immunisation can be shown in the absence of vaccination. See discussion.

For a number of years serious losses of baby pigs had been experienced on a large farm divided into several smaller holdings. On these premises it was the policy to receive pigs from the central farm and services were performed by a small stock of boars kept there. As a result, not only were sows on the holdings all of one breed, but also many were closely related. Histories showed that it was common for whole litters to be lost within a few days of farrowing, and although piglets appeared to be healthy at the time of birth, varying degrees of clinical jaundice became evident, generally at about the 48th hour, and it was usual for all pigs to be dead before the end of the first week. Following repeated failures to account for the condition by the usual methods of diagnosis, it was decided to explore the possibility of maternal iso-immunisation as the cause of the disease and opportunity arose to study litters of affected piglets, all of which were born of Wessex sows mated to Large White boars.

Symptoms

Clinical evidence of hæmolytic disease was seen originally in piglets of affected litters born on the farm and later was studied more closely under controlled conditions of incompatible mating. In the early field cases difficulty was experienced at times in obtaining a full account of the symptoms since on each occasion disease was well advanced and some members of affected litters had died by the time notification was received, which often resulted in the preliminary examination having to be made at night by poor light. However, later observations on litters in which the disease was expected enabled a fuller picture to be drawn and more precise knowledge to be gained on the variability of symptoms in different families.

In all cases jaundice was the most significant and characteristic symptom observed, and although it varied in its intensity, it was rarely absent in affected litters. In all cases piglets were born normally at full term and appeared to be healthy during the first 24 hours of life, after which symptoms developed rapidly. By the 48th hour, jaundice of the skin and membranes was plainly evident in the majority of cases and most animals showed weakness and lassitude to the extent of being unable to rise or be suckled. In piglets affected with the more acute form of the disease, deaths began on the second or third day. Subsequently, a small proportion of piglets recovered slowly over one to two weeks. In these animals there was a noticeable decrease in the degree of clinical jaundice coinciding with a fall in the rate of erythrocyte destruction by the maternal antibody, until only a mild anæmia was evident. It appeared that some pigs in the same litter enjoyed a greater degree of resistance to the effects of maternal antibody than others. In one litter, whilst some piglets had died at the fourth day, others displayed only mild anæmia and showed promise of recovery.

It is known that newborn mammals of some species can absorb antibody through the alimentary tract with great rapidity. In this way they may attain in a few hours a serum titre equalling that of the colostrum, but this absorptive capacity is transitory and often has ceased to exist after about 24 hours (Brambell *et al.*, 1951). It seemed probable, therefore, that the period might be limited

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during which baby pigs would absorb maternal iso-antibody from the alimentary tract and that such limitation might be an important factor in controlling the disease. Accordingly a litter from a sow which previously had engendered acute disease in her piglets was selected for further study. On this occasion the sow was mated to a different Large White boar. Five of the young were suckled from birth and the remaining five were removed and fed artificially. These were returned to the udder at intervals.

Piglets suckled from birth.

All five piglets were healthy at birth and their cord blood was negative to the antiglobulin sensitisation test, but all died from hæmolytic disease before the end of 60 hours and their red blood cells reacted positively to the antiglobulin sensitisation test during the period of clinical disease. The concentration of maternal antibody in the blood of the young pigs at the time of sampling was such that its agglutinating action on the red cells was clearly evident in the tube a few minutes after sampling, thereby reflecting the process of rapid erythrocyte breakdown going on in the body. It seemed certain that the piglets had obtained lethal quantities of maternal antibody from the sow's milk.

The piglets were kept under almost continuous observation from birth until the time of death. After the 18th hour of life they were sleepy and disinterested. At the 22nd hour three of them were very drowsy whilst the fourth, although dull, exhibited a greater degree of interest and movement. By the 36th hour two piglets were dead and the remainder were too weak to move or be suckled. The third pig died at the 40th hour after birth and the fourth and fifth at about the 56th hour. Of the five piglets, four presented a progressive clinical jaundice from the 24th hour of being suckled. The remaining pig died from a profound hæmolytic anæmia but at no time had exhibited jaundice. The blood of this pig showed very few red cells left in circulation and consisted almost entirely of plasma and free hæmoglobin resulting from an extremely rapid breakdown by the maternal antibody. Thus, apart from a single case of rapid fatal anæmia, the symptoms shown by the pigs suckled by the sow followed closely those observed in field cases of the disease in which jaundice predominated.

Piglets fed artificially for varying periods.

Five piglets were removed from the sow immediately after birth. These were fed artificially and were returned to the sow at different periods in an attempt to ascertain the minimum time during which piglets of iso-immunised sows must be deprived of maternal milk in order to survive.

The piglets were returned to the sow at six-hourly intervals after a minimum period of twelve hours' absence from the udder. Blood samples were taken at birth from the umbilical cords and afterwards at frequent intervals from the tails both before and after return to the sow. The red cells were subjected to the antiglobulin sensitisation test and were tested also against the mother's serum for reactivity against the maternal iso-antibody. In addition detailed hæmatological examinations were made upon piglets and these will be described in a later paper.

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All five piglets remained healthy during the period of artificial feeding and showed marked variation of symptoms following suckling. These piglets have been numbered from 6 to 10 for reference. Piglets 6 and 7 were returned to the sow after twelve hours and following six hours of being suckled maternal antibody was detectable in the blood of both. In spite of an appearance of progressive drowsiness, both were suckled and were able to move around the enclosure. From this time onwards piglet No. 7 developed symptoms typical of hæmolytic jaundice and died exactly 48 hours after return to the sow. During this time its blood was strongly positive to the antiglobulin sensitisation test. Piglet No. 6, on the other hand, passed through a stage of mild hæmolytic

TABLE I

Experimental litter of Sow 3
Rate of absorption of antibody

	Time							
Piglets	Birth	12 hrs.	18 hrs.	24 hrs.	36 hrs.	60 hrs.	8 days	26 days
1	-	+	+	+	D			
2	-	+	+	+	D			
3	-	+	+	+	+	D		
4	-	+	+	+	+	D		
5	-	+	+	+	+	D		
6	-	-	+	+	+	+		K
7	-	-	+	+	+	D		
8	-	-	-	+	+	+		K
9	-	-	-	+	D			
10	-	-	-	-	+	+	K	

Piglets 6 and 7 returned to sow at 12 hours.
Piglets 8 and 9 returned to sow at 18 hours.
Piglet 10 returned to sow at 24 hours.

— = negative to antiglobulin sensitisation test.
+ = positive to antiglobulin sensitisation test.
D = piglet died.
K = killed.

disease and recovered in spite of the fact that its blood contained large amounts of maternal antibody for 60 hours after having been suckled, but by the 84th hour its blood was negative to the antiglobulin test and, although weak, it appeared to be regaining health. It was killed when 26 days old, was in good condition and weighed 22 pounds.

Piglets 8 and 9 were returned to the sow after 18 hours. After five hours of being suckled neither pig exhibited symptoms, but both showed maternal antibody in their blood. After 20 hours with the sow both pigs were markedly positive to the antiglobulin test and erythrocyte clumping by maternal antibody could be detected in samples within a few minutes of bleeding. Piglet No. 8 maintained maternal antibody in its blood for three days and recovered, and

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during this time it experienced mild hæmolytic disease similar to piglet No. 6. It was killed after 26 days in fair condition at a weight of 18 pounds. Piglet No. 9, having shown a positive reaction to the antiglobulin test after five hours of being suckled, became ill rapidly and died of hæmolytic anæmia without jaundice within 17 hours of returning to the sow. Piglet No. 10 was not allowed to be suckled until 24 hours old; twelve hours later it seemed bright and alert, although its blood was now rich in maternal antibody. Within 40 hours, however, it showed marked clinical anæmia with mild jaundice and was killed in a state of extreme weakness, having been with the sow for six days.

Table I shows the rapidity with which antibody entered the bloodstreams of the piglets and produced agglutination of the red cells.

No evidence could be found of the presence of sensitised red cells in the blood of piglets before being suckled, thereby confirming that maternal antibodv had not traversed the placental barrier in quantities sufficient either to cause disease *in utero* or to be detected by the accepted methods of testing. Different pigs seemed to display variation both in their capacity to absorb iso-antibody from the digestive tracts and in the period over which absorption took place.

Post-mortem Findings

The post-mortem lesions of the disease, like the clinical symptoms, were divisible into those of anæmia with or without a variable degree of jaundice. In a high proportion of pigs, jaundice with profound anæmia was the commonest lesion encountered in both field and experimental cases of hæmolytic disease. In acutely icteric pigs the findings were fairly constant and consisted of discoloration of the skin and musculature from golden yellow to a deep "sun tan" brown. The most marked lesions were found in the viscera in all cases and the intestines exhibited jaundice. It was common to find enlargement and congestion of spleens, some of which were nearly black in colour, whilst livers often were congested and dark. Perhaps the most striking feature encountered, however, was the quantity of bloodstained peritoneal fluid which was present in nearly all carcases, which, like the darkened urine, was coloured by free hæmoglobin from broken-down erythrocytes and indicated plainly the hæmolytic nature of the disease. Lesions in other parts of the body, although frequently of diagnostic significance, in general were less striking than those of the abdominal cavity. In all cases the blood contained a greatly reduced number of erythrocytes and the plasma was often markedly yellow. In some cases there was noticeable subcutaneous œdema of the head region, in others the trunk was so affected, but these were not constant lesions at autopsy.

A different, but equally acute form of the disease consisted of profound and rapidly fatal anæmia. In such cases the musculature was of a milky whiteness and all organs were pale, except the spleen and kidneys which, as in the acutely icteric carcases, were congested. This form of the disease was less frequently seen, but nevertheless is undoubtedly a manifestation of hæmolytic disease in the pig.

Brain jaundice was encountered in the majority of pigs, the intensity of pigmentation varying with the degree of bodily jaundice. It was diffusely spread

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throughout the brain substance in the acute cases. Yellow pigmentation of the brain is often a complication of hæmolytic disease in the child, the effects of which may be far-reaching in later life (Pickles, 1949). Claireaux, Cole and Lathe (1953) were able to establish that the brain pigment in such cases consists of bilirubin. Cole and Lathe (1953) developed a chromatographic method of fractionating bile pigments and were able to show not only that the serum in such cases contained pigments other than bilirubin, but also that the brain tissue has a selective affinity for the extraction of bilirubin from the blood. Dr. G. H. Lathe of Queen Charlotte's Hospital, London, kindly undertook to examine brains and serum from affected animals for bile pigments. The results, which are given in Table II, show that although a number of pigments were detected in

TABLE II
Experimental litter of Sow 3
Bile Pigment Estimations

	Brain			Serum	
Pig	Description	Diazo Pigment mg./100 ml.	Type of Diazo Pigment	Diazo Pigment mg./100 ml.	Type of Pigment
1	Pale with faint generalized yellow stain	0.5	All Bilirubin	5.3	90% bilirubin Trace of pigment I
2	Pink without yellow appearance	0.44	All Bilirubin	2.9	All bilirubin
3	Pale with faint generalized yellow stain	0.44	All Bilirubin	5.4	90% bilirubin 10% pigment I Orange red pigment
4	Pale with faint generalized yellow stain	0.66	All Bilirubin	5.7	85-90% bilirubin 10-15% pigment I Faint orange red band
5	No apparent staining	0.73	All Bilirubin	5.3	50% bilirubin 40% pigment I 10% pigment II
7	Very pale and generalized yellow stain (more so than any above)	0.69	All Bilirubin	7.7	50% bilirubin 40% pigment I 10% pigment II

serum samples from six piglets, the brains had extracted bilirubin to the exclusion of all others, as occurs in the clinical cases in human infants.

In general, post-mortem findings presented evidence of icterus and acute hæmolytic anæmia associated with rapid destruction of erythrocytes.

Serology

The serological examinations have followed closely those undertaken in investigations of the syndrome in man and in other animal species. (Coombs, *et al.*, 1946, Caroli and Bessis, 1947.)

(i) *Examination of the serum of sows for iso-antibodies reactive against the red cells of the boar.*

The serum either unheated or after heating at 56° C. for half an hour, was examined for direct agglutinins. This was performed by adding a volume of a

2 per cent suspension of the boar's red cells to an equal volume of varying dilutions of the sow's serum. The red cells after sedimentation were drawn up in a pipette and examined on a glass slide under the microscope for agglutination. Where no agglutination was observed, the sera were further examined for the presence of incomplete antibodies by means of the indirect antiglobulin sensitisation test (Coombs, Mourant and Race, 1945). The rabbit anti-pig globulin serum had been absorbed previously with pig red cells and was therefore free of antibodies to these cells. The particular antiglobulin serum employed was used at a dilution of 1 in 10.

(ii) *Examination of the red cells of newborn piglets for evidence of in vivo iso-sensitisation by maternal antibody.*

Blood from piglets was drawn directly into acid citrate dextrose mixture in approximately equal parts. In the laboratory the red cells were examined by the direct antiglobulin sensitisation test (Coombs, Mourant and Race, 1946). For this test the red cells were washed four times in saline and then, as a 10 to 20 per cent suspension, were added to an equal volume of a 1 in 10 dilution of the rabbit anti-pig globulin serum on a glass tile. Agglutination indicates the presence of maternal antibody sensitising the cells. The cells of normal piglets are not agglutinated. In the following account the pertinent serological findings are recorded along with each case as described.

Sow 1. The first litter of seven piglets born to this sow was reared successfully but at the second farrowing all eleven of the young died within four days of birth from typical hæmolytic disease.

Blood from the piglets was not available for the direct sensitisation test. The sow's serum contained a direct agglutinin for the boar's cells at a titre of over 512. The cells after sensitisation with the maternal antibody were not lysed *in vitro* by either guinea-pig or pig complement at a dilution of 1 in 8.

Sow 2. This sow was a full sister to Sow 1. Two piglets of her second litter died within 24 hours of birth.

Blood samples from the two piglets were positive to the direct antiglobulin test and thus indicated iso-sensitisation. The serum of the sow tested twelve weeks later had a directly agglutinating antibody against the boar's cells to a titre of 8, but a titre of 128 was demonstrated by the indirect antiglobulin test.

Sow 3. This sow gave birth to three healthy litters before producing an affected litter. Her fourth family of eight were all severely affected within 24 hours of birth with clinical evidence of jaundice. All but three died within 42 hours of birth. Blood samples were tested from these three surviving piglets and all gave positive direct antiglobulin tests, while the cells of the sow and boar, acting as controls, were negative.

On testing the sow's serum against the boar's cells a direct agglutinin was found to a titre of 10 and an antibody demonstrable by the indirect sensitisation test to a titre of 640. This antibody had the characteristics of an incomplete antibody. Again the cells were not lysed *in vitro* by either guinea-pig or pig complement. The antibody in the milk on the day of farrowing showed a titre greater than 512.

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Sow 4. Disease appeared for the first time in the third litter of this sow. All thirteen pigs were alive four days after farrowing, but individuals showed great variation in the degree of anæmia and jaundice observed.

Blood was obtained from five piglets in this litter and all gave a positive direct antiglobulin test. The cells of the boar and sow, acting as controls, were negative. The sow's serum possessed direct agglutinins to the boar's cells to a titre of 256, but by the indirect sensitisation test the titre was over 512.

Experimental Litter of Sow 3. Cord blood samples were obtained from all the piglets and were negative to the direct antiglobulin sensitisation test. The bloods of all the piglets became positive over the course of the next 24 hours.

The direct agglutinins in the sow's serum had a titre of 16, while the titre by the indirect sensitisation test was over 1024. The antibody in the milk at this time showed a titre greater than 1024, but on the 27th day after farrowing it had fallen to less than 4.

Since the disease was first reported, over 250 samples of blood have been received from suspected cases of hæmolytic anæmia, and all but ten gave negative reactions with antiglobulin serum. These were shown to be new outbreaks of the disease.

From the serological point of view the findings were much as would be expected. Results obtained from the experimental litter support the evidence that the disease in the pig is strictly neonatal in that the maternal antibody does not pass through the placenta. The antibody in the serum of Sow 3 was of interest as it seemed to be of the incomplete type.

Hæmatology

Studies on the hæmatology of the disease were necessarily limited since many of the affected piglets were either dead or dying at the time of examination. With the survivors of one litter, however, it was possible to make restricted tests.

There was evidence which suggested that the speed of red cell destruction at times was so rapid that the organs responsible for the removal of cell breakdown products failed in their function and on occasion death supervened before jaundice became discernible. On the other hand, mild clinical cases of the disease showed correspondingly less anæmia and jaundice.

In blood samples from severely affected cases the red cells showed a tendency to undergo rapid hæmolysis and auto-agglutination in their own plasma at room temperature, within a few minutes of being taken, due to the presence of large quantities of maternal antibody in the circulating blood of the piglets. This reaction during the early acute stages of the disease made it impossible to do either accurate cell counts or hæmoglobin estimations as the latter were also influenced by the presence of abnormal amounts of bile pigments in the blood.

For hæmoglobin estimations the acid-hæmatin Sahli hæmoglobinometer was used with 100 per cent being taken as equivalent to 14 grammes of hæmoglobin per 100 c.c. of blood. Reticulocyte counts were based as a percentage of red blood cells and the nucleated red blood cells as a percentage of white cells, the total white cell count being known.

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The first cases to be studied were those from the litter of Sow 4, the piglets of which at four days old showed symptoms ranging from severe jaundice to mild anæmia. Blood samples from two severely jaundiced piglets showed approximate red blood cell counts of 500,000 to 750,000 per cmm. and a reticulocyte response of 38 per cent, while hæmoglobin, at less than 20 per cent failed to register on the instrument. Two of the less severely affected piglets were kept alive for a further 24 hours and samples of blood were examined at six-hourly intervals, but without any noticeable alteration in the picture of the red cells during the period. Both had red cell counts in the region of 1.1 millions per c.mm. and the hæmoglobin reading just registered at the 20 per cent mark. Blood smears showed that many red cells were ringed, enlarged and stained basophilic, while the reticulocyte counts were 60 per cent with normoblasts just over 40 per cent of 27,400 and 26,300 white cells.

An attempt was made to rear two of the least affected piglets on supplemented cow's milk. Both animals showed red cell counts in the region of 1.75 millions per c.mm. and hæmoglobin levels at 32 per cent when four days old.

Further samples were taken from one piglet on the 8th, 12th and 17th day. During this period the hæmoglobin level rose to 36, then to 40 per cent, but on the last reading dropped to 33 per cent. The red cell count rose more rapidly to 2.6 millions, then to 5 millions, but finally declined slightly to 4.6 millions per c.mm. The red cells were variable in size, mostly macrocytic and deficient in colour at first, but on the 12th day had become more normal in appearance. The reticulocytes present remained at about 15 per cent while the normoblasts ranged from 12 per cent (on 11,000 white cells) to 14 per cent (on 21,500 white cells).

The piglet was small but died unexpectedly when 18 days old.

The second piglet was reared artificially and did not grow adequately; when 8 days old it showed poorer blood readings than a survivor left on the sow, the red cell count being 0.5 million per c.mm. as against 1.2 millions, while both failed to register on the hæmoglobin scale. On the other hand, blood regenerative processes were very active as evidenced by a reticulocyte count of almost 80 per cent with normoblasts 47 per cent as compared with levels of 28 per cent and 22 per cent of the survivor on the sow. Both piglets died before any further samples could be taken.

Discussion

Hæmolytic anæmia and jaundice of the newborn may occur in some animal species and its ætiology is closely related to that of hæmolytic disease in man. Whilst there is no doubt that jaundice is the most easily detected symptom, it must be appreciated that a destructive anæmia constitutes the basis of the disease and this varies in its effects and depends upon the antibody titre of the mother. In women the picture is often complicated by the readiness with which antibody is able to attack the fœtus before birth, but in those species which obtain a passive immunity after birth the severity of attack is governed by the concentration of antibody in the colostrum and early milk as well as by the absorptive capacity of the digestive tract of the young animal. Thus, the condition *hydrops fœtalis*

has not yet been described in domestic animals in association with maternal immunisation, but high concentrations of antibody absorbed into the bloodstream of the newborn animal may evoke rapidly fatal anæmia without jaundice and cases have been seen in which subcutaneous œdema of the young pig accompanied hæmolytic disease. There is some support for the belief that in baby pigs the ability to absorb antibody begins to wane after 48 hours and that in a few days the blood regenerative processes are enabled to hold their own, although the sow's milk may still be rich in iso-antibody.

The circumstances permitting the iso-immunisation of sows are not yet known but from the evidence put forward by Goodwin, Saison and Coombs (1955) one factor is clearly the injection of animals with crystal violet swine fever vaccine which contains whole blood.

The possible sequelæ of the disease in pigs cannot yet be assessed, but it is probable that they are not so far-reaching as those of man. The finding in affected piglets of brain pigmentation due to bilirubin staining may be shown to be less significant than kernicterus of the human infant which can lead to spasticity and paralysis, resulting from permanent damage to the central nervous system. Nevertheless, the possibility of serious side-effects cannot yet be entirely discounted.

Summary

Observations have been made of cases of hæmolytic disease of newborn pigs caused by maternal iso-immunisation.

All litters were healthy at birth and little change was observed during the first 24 hours. Thereafter the clinical picture varied considerably in the degree to which litters and individual members were affected. All of some litters were dead within 48 hours of birth and showed marked jaundice. Others had individuals severely affected with jaundice when four days old, with the remaining members exhibiting varying degrees of anæmia only. In a few cases piglets recovered from the disease.

Anæmia was present on all occasions and the speed of red cell destruction by maternal antibody seemed to determine the degree of jaundice observed in the skin, musculature and liver at autopsy. There was enlargement of the spleen accompanied often by bloodstained peritoneal fluid and hæmoglobinuria. In some cases the brain was diffusely stained with bilirubin. The milder cases showed anæmia with less jaundice and few macroscopic changes of the internal organs.

The disease was diagnosed serologically using the techniques employed for investigations of the syndrome in man and equines. Some sows had direct agglutinins against the boar's red cells, whilst in others the presence of incomplete antibody was demonstrated by means of the indirect sensitisation test. It was shown that maternal antibody was not present in the blood of the fœtus in detectable amounts before it was suckled but it was concentrated in the colostrum and its absorption from the alimentary tract of the young pig was sufficiently rapid for its presence to be demonstrated in the blood, often in great quantity, a few hours after the first time it had been suckled.

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Hæmatological results conformed closely with the clinical picture and showed hæmoglobin loss corresponding with the degree of anæmia which was present. When symptoms suggested that the animal was making some recovery from the disease the blood regenerative centres were very active and large numbers of normoblasts and reticulocytes were found.

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MARROW BIOPSY IN SHEEP—II. ANÆMIA

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Introduction

HOLMAN (1945) has shown that as most anæmias in sheep are normocytic, it is not possible to make the same use of the blood indices in their classification as in man. He suggested a classification into post-hæmorrhagic, hæmolytic and dyshæmopoietic similar to that of Vaughan (1936). To classify anæmias in this way from the examination of the peripheral blood alone, great reliance is placed on the presence or absence of regenerative forms. However, Wirth (1938) has shown that in the sheep the signs of regeneration appearing in the peripheral blood in response to a stimulus such as blood loss, are not marked and quickly disappear once the stimulus is removed. Thus the simultaneous examination of the blood and bone marrow may often be necessary in the study of anæmic states in sheep.

The work to be described was carried out to test this hypothesis and consists of a study of the blood and bone marrow changes in eleven sheep. In three of the sheep the anæmia was experimentally induced by blood loss and in the remaining eight existed as a symptom of general unthriftiness.

Materials and Methods

Three two-year-old Cheviot sheep were used for the study of anæmia produced by venesection. They were numbered V.35, E.63 and N.50. From

sheep V.35, 600 ml. of blood was withdrawn on the first day and there was no further bleeding. In sheep E.63 and N.50, blood was removed over a period of 8 and 12 days respectively. The amounts withdrawn and the days on which bleeding took place are shown in Tables II and III. The techniques used for

EFFECT OF BLOOD LOSS ON PERIPHERAL BLOOD

TABLE I — SHEEP V.35

600 ml. blood withdrawn on day 0.

Day.	0	1	2	3	4	5	43
Erythrocytes.							
R.B.C. 10^6/cu.m.	11.8	8.9	7.1	7.2	6.8	9.1	10.1
M.C.V. cu.μ.	36.0	36.0	38.0	38.5	41.0	34.0	41.0
M.C.H.C. %	28.5	27.0	29.5	30.0	30.0	28.0	30.0
Reticulocytes %	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cells showing Polychromasia %	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cells showing Punctate Basophilia	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Leucocytes/cu.mm.							
Neut. Polymorphs	260	1,092	847	868	863	60	840
Eosinophils	260	1,326	385	620	150	240	112
Basophils	65	480	154	248	113	180	0
Lymphocytes	5,460	4,836	6,006	4,216	5,888	5,280	4,144
Monocytes	455	546	308	248	488	240	504

TABLE II — SHEEP E.63

Day	0	1	2	3	4	5	6	7	8	9	12	13	18	20	69
Blood withdrawn (m.l.) *	400	325	500	580	420	500	0	150	0	0	0	0	0	0	0
Erythrocytes															
R.B.C. 10^6/cu.mm.	9.9	8.3	7.5	7.5	5.5	6.9	4.9	4.4	4.5	4.8	6.0	6.4	7.6	9.0	11.5
M.C.V. cu.μ.	40.0	41.4	43.0	38.0	45.0	35.0	36.0	40.0	37.0	37.5	39.0	39.0	43.0	45.0	31.0
M.C.H.C. %	30.0	27.0	28.5	29.0	31.0	29.5	29.5	30.0	26.5	27.5	25.5	28.0	30.0	28.0	30.0
Reticulocytes	0.0	0.0	0.0	0.0	0.65	0.60	0.75	0.51	0.25	0.30	0.0	0.0	0.0	0.0	0.0
Erythrocytes Polychromasia %	0.0	0.0	0.0	0.0	0.03	0.21	0.52	0.36	0.85	0.31	0.04	0.02	0.0	0.0	0.0
Erythrocytes showing Punctate Basophilia %	0.0	0.0	0.0	0.0	0.03	0.28	0.12	1.71	2.75	1.86	0.63	0.01	0.01	0.0	0.0
Leucocytes / cu.mm															
Neut. Bands	0	0	0	0	0	31	0	0	0	0	0	0	0	0	0
Neut Polymorphs	1,155	1,560	1,014	578	1,764	1,209	1,794	1,377	1,566	1,334	1,014	504	1,175	1,403	1,564
Eosinophils	655	520	468	306	189	403	104	306	116	184	104	80	94	83	561
Basophils	70	40	78	170	32	124	0	0	0	92	0	0	0	27	30
Lymphocytes	4,655	5,560	6,045	5,508	4,221	4,185	4,865	3,213	3,828	2,668	3,666	4,611	3,149	3,300	3,186
Monocytes	455	320	195	238	95	248	138	204	290	322	416	106	282	688	561

* On each occasion blood withdrawn after sample collected.

the collection and examination of blood and marrow samples have been described (Grunsell, 1951 and 1955*a*). The times of blood sampling are shown in Tables I, II and III and of marrow sampling in Figs. 1, 2 and 3.

The eight sheep showing clinical signs of anæmia were representative of a

form of chronic debility which was causing recurrent losses in the three flocks from which they came. Before slaughter and autopsy, samples of blood and bone marrow were examined, using the techniques already mentioned. The results of the peripheral blood examination were assessed by comparison with figures obtained from hill sheep sampled in January (Grunsell, 1955*b*). The standards used for the marrow results were determined from the examination of the marrow of ten normal sheep (Grunsell, 1951). At the time of sampling all eight sheep were found to be grossly emaciated.

TABLE III — SHEEP N.50

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	14	31	41
Blood withdrawn (c.c.)*	780	780	0	250	250	250	0	250	250	250	0	250	0	0	0	0
Erythrocytes																
R.B.C. 10^6/cu.mm.	13.3	10.4	8.8	8.1	7.7	7.0	6.6	6.6	6.0	6.3	4.0	5.1	6.1	7.3	11.3	14.2
M.C.V. cu μ.	35.5	38.0	36.0	34.5	35.5	36.0	38.0	38.0	41.5	42.0	41.5	35.0	33.5	33.0	32.0	28.5
M.C.H.C. %	27.5	28.0	27.0	29.5	28.5	28.5	28.5	25.5	25.5	25.5	27.0	27.0	27.0	29.0	27.0	30.5
Reticulocytes %	0.0	0.0	0.0	0.0	0.80	0.64	1.14	0.55	0.46	0.55	0.40	0.22	0.57	0.07	0.0	0.0
Erythrocytes Showing Polychromasia %	0.0	0.0	0.0	0.0	0.06	0.18	0.58	0.14	0.08	0.18	0.0	0.03	0.05	0.0	0.0	0.0
Erythrocytes Showing Punctate Basophilia %	0.0	0.0	0.0	0.0	0.80	0.75	2.02	0.54	0.51	1.28	0.91	0.64	1.56	0.22	0.0	0.0
Leucocytes																
Neut. Polymorphs	1,624	1,660	950	1,558	1,318	1,030	456	1,063	1,628	1,269	1,064	1,176	1,716	340	742	3,146
Eosinophils	1,276	1,265	1,188	979	553	824	532	468	945	658	456	588	440	425	424	1,392
Basophils	58	0	0	45	85	52	0	43	0	0	76	42	0	0	0	0
Lymphocytes	1,178	8,580	7,030	5,830	6,333	7,725	6,232	6,545	7,508	7,003	5,662	6,174	6,292	7,225	9,116	6,655
Monocytes	464	195	333	490	213	670	380	383	420	470	342	420	352	510	318	908

* On each occasion blood withdrawn after sample collected.

Results

(*a*) *Experimental Anæmia.*

The changes in the blood picture of the three sheep are shown in Tables I (V.35), II (E.63) and III (N.50). A summary of the differential marrow counts and the cellularity estimations is illustrated in Figs. 1 (V.35), 2 (E.63) and 3 (N.50). The Figures also include myeloid erythroid ratios, percentages of cells seen in mitosis and maturation curves.

The erythrocyte count was reduced in the case of V.35 to 57.6 per cent of the prebleeding level. In E.63 and N.50, the reduction was to 44.4 and 30.0 per cent respectively. The corpuscular indices showed a normochromic normocytic anæmia in the case of V.35 and E.63, hypochromic macrocytic in N.50. Anisocytosis and poikilocytosis were seen in the films from all three sheep when the erthrocyte levels were at their lowest points. Immature erythrocytes were found in the peripheral blood as cells showing punctate basophilia and polychromasia, and as reticulocytes and late normoblasts. Regenerative forms did not appear until erythrocyte levels had been reduced by 50 per cent in the case of E.63 and 39 per cent in sheep N.50. The incidence declined sharply once bleeding was stopped. Punctate basophilia was more prevalent and persisted longer than the other forms of regeneration. It was the only sign of regeneration in films from V.35. The highest incidence was 2.75 per cent in E.63 on day 8. The

incidence of reticulocytes and polychromatophilic cells was much lower, the latter being much less numerous than the former. When supravitally stained films were counter stained with Leishmann and Giemsa no cells showing polychromasia were found. This suggests that at least some of the erythrocytes showing as reticulocytes in supravital preparations, appeared in the films stained by Leishmann and Giemsa as polychromatophilic. Late normoblasts were only seen on days 4, 6, 7 and 8 in E.63 and on day 7 in N.50.

Figs. 1, 2 and 3 show that there was an increase in the cells of the marrow in response to the blood loss and that this increase was mainly due to an erythroid hyperplasia. Coinciding with the hyperplasia in all three sheep there was a shift to the left in the erythroblasts which took the form of an increase in proerythroblasts and early normoblasts. The rise in the percentage of cells in mitosis which accompanied the hyperplasia was mainly the result of greater mitotic activity in the intermediate normoblasts.

In sheep V.35 the numbers of neutrophilic leucocytes rose 24 hours after bleeding. The fluctuations in the levels of these cells in the other sheep could not be related to the experimental interference. In the granuloblastic tissue the only response to blood loss was in sheep N.50, in which there was a shift to the left in the neutrophilic series on day 7 (Fig. 3). The eosinophilic leucocyte count was higher in V.35 and N.50 than in E.63. This difference was reflected in the marrow and whereas in E.63 lobulated eosinophils predominated, in V.35 and N.50 the eosinophilic myelocytes and metamyelocytes generally outnumbered the lobulated cells.

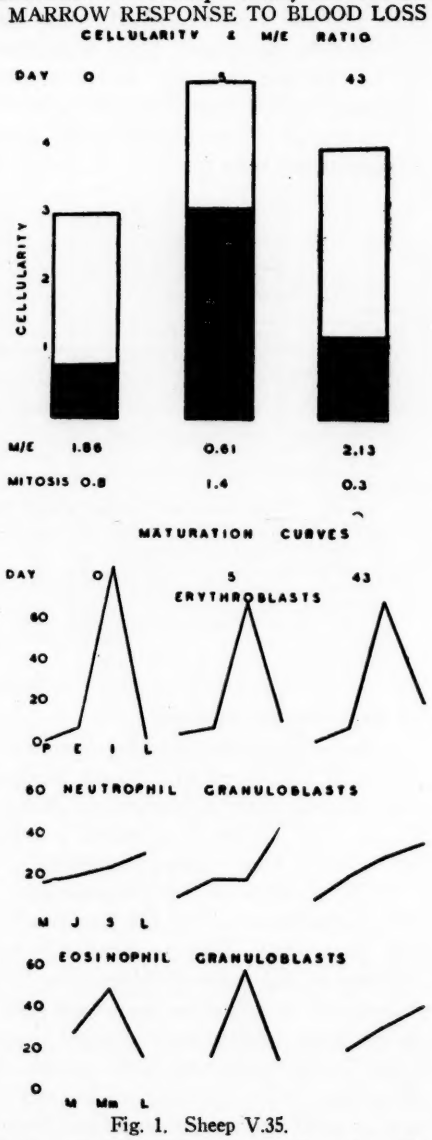

Fig. 1. Sheep V.35.

(*b*) *Sheep showing clinical anæmia.*

A summary of the blood pictures and hæmomyelograms of the eight sheep showing clinical signs of anæmia is contained in Table V. From this data the

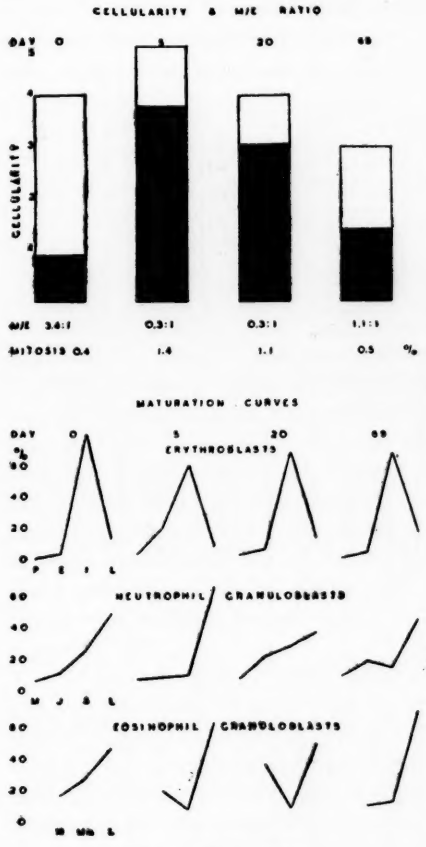

Fig. 2. Sheep E.63.

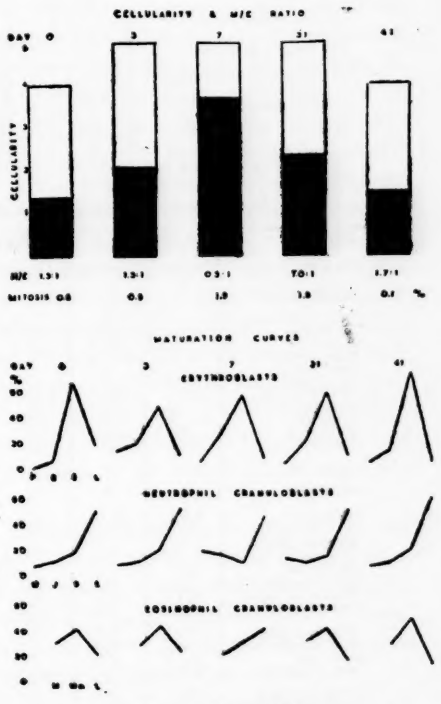

Fig. 3. Sheep N.50.

Key to Figs. 1, 2 and 3

Granuloblasts and other cells.
Erythroblasts

M/E — Myeloid Erythroid Ratio.
P — Proerythroblasts.
E — Early Normoblasts.
I — Intermediate Normoblasts.
L — Late Normoblasts.
M — Myelocytes.
Mm — Metamyelocytes.
J — Juvenile Metamyelocytes.
S — Stab Metamyelocytes.
L — Lobulated cells.

XUM

anæmias were classified as either hyperplastic or hypoplastic or aplastic. The classification was supported by the examination of sections of marrow from various parts of the body, post-mortem.

TABLE IV. Mitosis in Erythroblasts

Sheep No.	V. 35			E. 63				50				
	Day			Day				Day				
%	0	5	43	0	5	20	69	0	3	7	31	41
Proerythroblasts	-	0.2	-	-	-	0.1	0.1	0.1	0.1	-	-	-
Early Normoblasts	-	0.1	-	-	0.2	0.2	-	0.1	0.1	0.2	0.1	-
Intermediate Normoblasts	0.6	1.0	0.1	0.1	0.6	0.7	0.2	0.3	0.1	1.4	1.1	0.1
Late Normoblasts	-	-	-	-	0.2	0.1	-	-	-	0.1	-	-
Total	0.6	1.3	0.1	0.1	1.0	1.1	0.3	0.5	0.3	1.7	1.2	0.1

Table V. Haemomyelograms of eight sheep showing clinical signs of anaemia.

Sheep' No.	H.602	L.20	H.613	99/51	51	45	H.911	R.200
Age & Sex	2 y.o.f.	2 y.o.f.	2 y.o.f.	2 y.o.f.	8 m.o.m.	8 m.o.m.	5 y.o.f.	10 y.o.m.
Peripheral Blood								
R.B.C. 10^6/cu.mm.	1.7	3.1	3.5	6.2	5.3	6.5	9.4	4.2
M.C.V. cuu.	51.0	41.0	37.5	31.0	35.5	35.0	30.0	31.0
M.C.H.C. %	23.5	28.5	21.5	29.0	33.0	28.0	29.5	30.0
Regeneration	++	-	-	-	-	-	-	-
Neut. Juv.	0	0	0	0	30	0	0	0
Neut. Band	2,090	0	71	88	1,131	16	793	745
Neut. Polymorphs.	15,234	2,593	6,035	6424	9,223	1,914	4,865	12,814
Eosinophils	0	0	71	0	0	0	0	0
Basophils	0	0	0	0	0	16	0	0
Lymphocytes	2,388	5,865	639	1760	1,250	1,287	1,139	1,043
Monocytes	100	35	284	528	268	66	104	198
Marrow								
Cellularity	V.	V.	V.	II.	IV.	0	0	0
M/E Ratio	4.6 : 1	0.2 : 1	1.4 : 1	15.0 : 1	37.3 : 1	A	A	A
Maturation Curves						C	C	C
%						E	E	E
Proerythroblasts	4.2	6.3	1.2	6.6	0			
Early Normoblasts	3.6	11.8	10.2	8.3	8.7	L	L	L
Intermediate Norms.	65.1	73.1	82.2	61.7	82.6			
Late Normoblasts	27.1	8.7	6.3	23.3	807	L	L	L
Neut. Myelocytes	15.8	5.2	14.4	14.1	25.0			
Neut. M/myelocytes	12.7	5.2	35.6	20.0	25.6	U	U	U
Neut. Band	23.1	27.8	25.1	21.5	33.2			
Neut. Polymorphs.	48.4	61.7	24.9	44.4	16.2	L	L	L
Eosin. Myelocytes	22.2	13.8	28.1	24.3	27.7			
Eosin. M/myelocytes	42.6	37.9	56.2	31.1	23.2	A	A	A
Eosin. Polymorphs.	35.2	48.3	15.6	44.6	49.1			
						E	E	E
Classification of Anaemia.	Hyper-plastic	Hyperplastic with arrest of maturation.		Hypoplastic		Aplastic		

Discussion

The results show that the response of the marrow to a stimulus such as blood loss can be demonstrated by marrow biopsy. The changes in the marrow agreed with those described in post-hæmorrhagic and hæmolytic anæmia in man (Israels, 1948). In the peripheral blood the immature erythrocytes showing punctate basophilia outnumbered polychromatophilic and reticulated cells, which confirms the findings of Holman (1945) and Wirth (1938), and shows that regeneration can be estimated in the sheep on the presence of punctate basophilia

alone, supravital staining to demonstrate reticulocytes being unnecessary. A blood loss reducing erythrocyte levels to half the original in six days (E.63 and N.50) constitutes a severe stimulus and one likely to result in the appearance in man of up to 50 per cent immature cells in the peripheral blood (Castle and Minot, 1936), but in no sheep in this investigation did the total of all three immature forms expressed as a percentage exceed 3.8. Furthermore, although signs of regeneration in man usually persist for up to 14 days after hæmorrhage has ceased, in E.63 and N.50 regenerative forms had disappeared in 11 and 3 days respectively after the last bleeding. Wirth (1938) has reported similar findings in sheep. On the other hand, the marrow changes persisted after the regenerative forms had disappeared from the peripheral blood. These results indicate that before an aregenerative anæmia in the sheep can be classified as hypoplastic it is necessary to carry out an examination of the marrow.

The advanced hypochromic macrocytic anæmia in sheep H.602 was accompanied by regenerative changes and a neutrophilia in the peripheral blood. The marrow showed a high cellularity with a preponderance of granuloblasts and a shift to the left in the erythroblasts. A heavy infestation of *Hæmonchus contortus* was found in this sheep post-mortem.

In both L.20 and H.613 the anæmia was normocytic. In L.20 it was normochromic and in H.613 hypochromic. Although there were no regenerative signs in the peripheral blood of either sheep, they both showed a hyperplastic marrow and in L.20 the hyperplasia was predominantly erythroblastic. These findings in the presence of the pronounced anæmia existing in the sheep suggest an arrest in the maturation in the erythroblasts as the cause of the anæmic states. There were no significant post-mortem findings in the case of sheep L.20, but H.613 was found to be carrying a heavy infestation of *Ostertagia* and *Trichostrongylus* spp.

The aregenerative normochromic normocytic anæmia in 51 and 99/51 was accompanied in the marrow by a hypoplasia of the erythroblastic tissue with a shift to the left in the erythroblasts in sheep 99/51. This sheep was found to have Johne's disease and advanced fascioliasis and to be carrying heavy infestations of *Ostertagia* and *Trichostrongylus* spp. Sheep 51 had a heavy worm burden which was predominantly *Bunostomum trigonocephalum*.

In the three remaining sheep there was an aplasia of all marrow tissue. In 45 and R.200 this was reflected in the peripheral blood by a normochromic normocytic aregenerative anæmia. In H.911 the blood picture was normal. Autopsy showed sheep 45 to be carrying a light mixed worm infestation; in R.200 there were no significant findings beyond advanced cachexia, and in H.911 there was evidence of Johne's disease and a heavy infestation of *Ostertagia* and *Trichostrongylus* spp.

In sheep H.602 it is probable that the anæmia was due to the blood-sucking properties of *Hæmonchus contortus*, in that the blood and bone marrow findings confirm those of Fourie (1931) in anæmia due to hæmonchosis. No conclusions as to the ætiology of the anæmic states in the remaining sheep can be drawn because of the variation in the post-mortem findings, but the presence of heavy

worm burdens in four of the seven sheep is of some interest in view of the influence even subclinical helminthiasis has been shown to exert on the blood picture of sheep (Grunsell, 1955*b*). The anæmia in these seven sheep may be classified as dyshæmopoietic, the differing pictures merely representing stages in a terminal exhaustion of the hæmopoietic tissue.

Conclusions

Response of the hæmopoietic tissue to hæmorrhage is best seen in the bone marrow. Marrow biopsy is of considerable value in studying anæmia in sheep.

Acknowledgments

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THE ERYTHROCYTE SEDIMENTATION RATE IN NORMAL CATTLE AND IN CATTLE INFECTED WITH *MYCOBACTERIUM JOHNEI*

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Introduction

Since Fahraeus (1918) first noted the variation in suspension stability of human blood in pregnancy and in disease, a considerable study of this phenomenon has taken place. As a result, a normal value for the rate at which the corpuscles settle in a column of blood has been established for man. This value has been called the *Erythrocyte Sedimentation Rate* (E.S.R.).

In some diseases the E.S.R. is altered. This variation in E.S.R. is non-specific and its principal usefulness in human medicine is to distinguish between organic and psychotic disease. If the E.S.R. is increased the clinician should suspect an organic cause.

The E.S.R. has also been used extensively in the study of pulmonary tuber-

culosis, not as a diagnostic test but as an indication of the progress of the disease during therapy.

In the field of clinical veterinary medicine the figures given for the E.S.R.s of the domestic animals (Coffin. 1952) suggest that the usefulness of the test might be restricted to the horse, dog, and perhaps the pig. Ferrando (1938) found the test useless in the cow and Ferguson (1953) introduced a modification later which depended on reading the fall at the seventh hour. More recently, Bunce (1954), also working with bovine blood, has given figures for readings at intervals up to 24 hours.

The present paper gives the results for the erythrocyte sedimentation rate of cattle infected with *Mycobacterium johnei* and provides further evidence that this test is useless in the clinical examination of cattle.

Method

Various methods of carrying out the test are in general use. The Westergren was selected because the column of blood is taller in this method than in the others and any error due to packing of the cells is likely to be minimised.

TABLE I

Erythrocyte sedimentation rate of normal cattle.
(Figures are in millimetres)

	HOURS					
	1	2	3	4	5	24
Cow	0	0	0.75	1	1	8
"	0	1	1	1.5	2.5	10
"	0	0	1	1.5	2	9
"	0	0	0.75	1	2	11
"	0	0	0.5	1.5	2.5	12
"	0	0	1	1	2	10
Calf	0.5	1	1.5	2.5	3	14
"	0.5	1.5	1.5	3	5	21
"	0	1	1.5	2	2.5	13
"	0.5	1	2.5	2	2.5	10
"	0.5	1	1.5	1.5	2	10
"	0.5	1	2	2	2.5	9
"	0	1	2	2	2.5	14
"	0.5	0.5	1.5	2	2	9
"	0.5	1	2	2	2.5	10
"	0.5	0.5	1	1	2	10
"	0.5	1	1.5	1.5	2	8
"	0.5	1	1	1.5	2	6
Mean Values	0.3	0.7	1.3	1.7	2.3	10.8

TABLE IV

Erythrocyte sedimentation rate of cattle infected with Mycobacterium johnei
(Figures are in millimetres)

	HOURS					
	1	2	3	4	5	24
Bullock	0.5	1	1	1.5	1.5	7
"	0.5	1	1	1.5	2	12
"	0.5	1	1	1.5	2	7
"	0.5	1	1	2	2.5	11
"	0.5	0.5	1.5	2	2.5	14
"	0.5	1	1.5	2	2.5	11
"	0.5	0.5	1	1.5	2	10
"	0.5	1	1.5	2	2.5	12
"	0.5	1	1.5	2	2.5	11
"	0.5	1	1	1.5	2	9
"	0.5	1	2	2.5	3	15
Heifer	0.5	1	1.5	2	2.5	13
"	1	1.5	2.5	3	4.5	23
"	0.5	1	1	1.5	2	9
"	0.5	1	1.5	1.5	2	10
"	0.5	1	1.5	1.5	2	10
Mean values	0.5	0.9	1.4	1.8	2.4	11.5

Blood was collected from the jugular vein and mixed with a 3.8 per cent solution of sodium citrate in the proportion of 4 parts blood to 1 part solution. As soon as practicable (15 minutes to one hour) after collection, a sample of the citrated blood was drawn into the Westergren tube to the "O" mark. The tube was then placed vertically in its rack and the length of the clear column of fluid

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above the corpuscles was measured in millimetres at intervals of 1, 2, 3, 4, 5, and 24 hours.

Results

Normal Cattle.

The first series of tests was made on normal cattle, all of which were free from tuberculosis and Johne's disease; there were six 4-year-old cows and twelve four to 8-month old calves. Sedimentation was minimal within the first few hours and even at 24 hours the readings were not high. The results are given in detail in Table I.

Cattle Infected with Mycobacterium johnei.

The test was next applied to a group of eleven bullocks and five heifers, varying in age from 6 months to 1 year. Each of these animals had been infected with *Mycobacterium johnei* in calfhood and, although none of them was affected with clinical Johne's disease, all were excreting the organism intermittently in the fæces.

The results are given in Table II, from which it will be seen that there is no difference in the sedimentation rates of this group and those of the normal cattle in the previous group.

Clinical Johne's Disease.

Among a number of bullocks which had been infected with *Mycobacterium johnei* as calves were two which were showing symptoms of Johne's disease, and the opportunity was taken to test their bloods. Tests were made at approximately weekly intervals for five weeks before their deaths from Johne's disease. In neither case did the test give any indication that the disease was progressing towards a fatal termination. On the contrary, the results are comparable with those of the normal cattle. (See Table III.)

TABLE III

Erythrocyte sedimentation rate of cattle dying of Johne's disease
(Figures are in millimetres)

Weeks before death	HOURS 1	2	3	4	5	24
BULLOCK I						
5	0.5	1	1	1.5	2	7.5
4	0	0.5	1	2	2.5	10.5
3	0.5	1	1	1.5	2	9
2	0	1	1	2	2.5	10
1	0.5	1	1	1.5	2	7
BULLOCK II						
5	0.5	1	2	2.5	3	11.5
4	0	1	1.5	2	2.5	12
3	0.5	1	1.5	2	2.5	9.5
2	0	1	1	2	2	9
1	0	1	1	1	1.5	7

Discussion

Bunce used Wintrobe tubes to measure the E.S.R., whereas here we are considering figures obtained by using Westergren tubes. Nevertheless it is clear that, considering the limit of accuracy of either method, the early readings given for normal cattle do not differ from hers. At the 24-hour period, however, the readings are more than three times those quoted by her. This is largely because the column in the Westergren tube is double that of the Wintrobe tube and at 24 hours the measurement is no longer one of sedimentation rate but rather of the packing effect of gravity on separate cells suspended in plasma.

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The true sedimentation rate depends on the tendency of erythrocytes to form rouleaux (Ratner, 1943) or aggregates. These aggregates have a large volume but relatively small surface area, and therefore fall more rapidly. Any increase in rouleaux formation will therefore result in an increase in E.S.R. The reasons for the increase in rouleaux formation are not fully understood, but may depend on an alteration of surface charge of the red blood cells, which in turn is the result of some obscure action of the plasma proteins.

In the ruminant there is little or no tendency to form rouleaux (Holman, 1955) and therefore any suggestion of the establishment of a normal E.S.R. for cattle is misleading. Admittedly bovine erythrocytes do sediment in a column of blood, but it is not this slow settling of individual corpuscles that the erythrocyte sedimentation test is intended to measure. The test is designed to measure the much more rapid fall of large aggregates of corpuscles; these aggregates occurring either normally or as a result of alteration in the plasma because of disease.

Where an increase in sedimentation rate does occur in cattle, it can usually be associated with a low hæmoglobin value, as can be deduced from the figures given by Greatorex (1954).

Ferguson waited for seven hours before reading his tests and Bunce has said that, as the E.S.R. in cattle is small, "it is necessary to let the blood stand for 24 hours . . . before readings are taken if this test is to be of any practical significance." For the reasons stated above, a 24-hour reading will have no significance, and the significance of even a 7-hour reading is open to question. To be of any clinical value the E.S.R. must be measurable within the first hour or two of the collection of the sample (Wintrobe, 1951).

Summary

Experimental infection of cattle with Johne's disease did not increase the Erythrocyte Sedimentation Rate even in advanced clinical cases.

For reasons stated, standards for the E.S.R. of normal bovine blood cannot be formulated.

Conclusion

The Erythrocyte Sedimentation Rate test is unlikely to have any application in bovine clinical medicine.

Acknowledgment

I should like to thank Dr. H. H. Holman for his guidance during the preparation of this paper.

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STUDIES IN BOVINE MALIGNANT CATARRH

VI. Adaptation to Rabbits†

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REFERENCE has been made in a previous article (Piercy, 1953) to the failure of all methods investigated to preserve the virus of Bovine Malignant Catarrh (B.M.C.) for longer than a few days. The successful adaptation of the organism to small laboratory animals becomes all the more important, therefore, since otherwise the only available means of experimental study is by the expensive procedure of successive transmissions through cattle.

All attempts to infect fertile eggs, mice and guinea-pigs failed. Daubney and Hudson (1936) claimed successful transmission to mice and guinea-pigs but no details were given. The same authors quote the unsuccessful attempts of other workers (Goetze and Liess, 1929) to infect mice, rats, guinea-pigs, cats, dogs and pigs, whilst Mettam (1923) stated that " horses, sheep, dogs, fowls and the ordinary laboratory animals are naturally immune and unaffected by large inoculations of virulent blood."

It was Daubney and Hudson (1936) who first produced unequivocal evidence that the rabbit may successfully be infected with certain strains of B.M.C. virus. Moreover, they were able to reproduce a typical fatal form of the disease in cattle given brain from rabbits of the third passage. The lapinised strain was carried through a total of 28 passages (Daubney, 1937).

Pattison (1946) records the successful transmission of a Palestinian strain of the " head and eye " form of B.M.C. to three out of nine rabbits inoculated intraperitoneally with 5 ml. of whole blood taken from reacting cattle. Sub-inoculations beyond the second rabbit passage were unsuccessful.

During the course of the work reported in this article, four different series of rabbits were infected successfully from cattle reacting to B.M.C.

Materials and Methods

Rabbits. A total of nearly 300 rabbits of mixed breeds, usually four to eight months old, was used in these experiments. From time to time intercurrent diseases such as coccidiosis and pasteurellosis confused the issue by causing early deaths and regular dosing with sulpha drugs became a necessary routine.

Virus Strains. Although other strains were tried, successful results were obtained only with the " D " strain of B.M.C. (Piercy, 1952), maintained for three years in cattle at the Kabete laboratories for over forty generations.

Technique. Material for subinoculation was usually taken from rabbits destroyed when evidence of impending death was obvious. Tissues such as gland, brain and spleen were macerated in a mortar containing a little sterile sand and

† This is the final article in this series.

* This work was carried out at the Veterinary Research Laboratories, Kabete, when the author was a member of the Kenya Department of Veterinary Services.

mixed with sufficient saline or nutrient broth to make about a 10 per cent suspension. Unless otherwise stated, all subinoculations were done within an hour or two of harvesting, using unfiltered material. Blood was collected into citrate solution.

TABLE I.

BOVINE MALIGNANT CATARRH - RABBIT PASSAGES

Rabbit No.	Passage No.	Material and route.	Incubation period days PI	Death days PI	Remarks
SERIES I.					
1	1	1.0 ml gl. ex bovine 60 i/c	27	29	Nervous symptoms pronounced.
2	2	" br. ex rabbit 1 "	25	30	No nervous symptoms. Diarrhoea. Ocular and nasal discharges.
3 - 4	3	0.5 ml br. " " 2 "	14	18	ditto. ditto. ditto.
5 - 8	4	" br. " " 3 "	10 - 15	17-25	ditto. ditto. ditto.
		Lost at passage 5.			
SERIES II.					
65	1	1.0 ml gl. ex bovine 1894 i/c 0.5 ml " " " "	-	-	Died 14th day. Cause unknown.
66	1	" " " " "	-	-	No reaction. Discontinued.
67	1	" " " " "	19	26	Diarrhoea. Ocular and nasal discharges.
68	1	" " " " "	18	20	ditto. ditto. ditto.
69	2	2.5 ml sp. ex rabbit 67 i/p	8	14	ditto. ditto. ditto.
		Lost at passage 4.			

TABLE I (Cont'd.).

BOVINE MALIGNANT CATARRH - RABBIT PASSAGES.

Rabbit No.	Passage No.	Material and route.	Incubation period days PI	Death days PI	Remarks.
SERIES III.					
77	1	1.0 ml gl. ex bovine 1873 i/c 0.5 ml " " " i/p	16	18	Diarrhoea. Ocular & nasal discharges.
78	1	" " " " "	15	15	ditto, ditto. ditto.
79	1	0.5 ml " " " i/s	14	17	ditto, ditto, ditto.
80	1	" " " " "	-	-	Died other causes.
81	1	1.0 ml " " " i/c 0.5 ml " " " i/p	16	18	Diarrhoea. Ocular & nasal discharges.
82	1	" " " " "	-	-	No reaction. Discontinued.
83	2	1.5 ml sp. ex rabbit 81 i/c 0.25 " " " i/p	- -	- -	ditto. ditto.
84	2	" " " " "	41	45	Ocular & nasal discharges.
85	2	" " " " "	52	55	ditto, ditto.
86	2	" " " " "	25	27	Diarrhoea. Ocular & nasal discharges.
87	3	2.0 ml sp. " 86 i/p	11	14	ditto, ditto, ditto.
88	3	" " " " "	11	14	ditto. ditto. ditto.
		Lost at passage 11.			

TABLE I (Cont'd.).

BOVINE MALIGNANT CATARRH - RABBIT PASSAGES.

Rabbit No.	Passage No.	Material and route.	Incubation period days PI	Death days PI	Remarks.
SERIES IV					
1	1	2.0 ml gl. ex bovine 2800 s/c 1.0 ml " " " i/p	-	-	No reaction. Discontinued.
2	1	0.5 ml " " 2822 i/c 1.0 ml " " " i/p	-	-	ditto. ditto.
3	1	0.25 " " 2865 i/c 1.0 " " i/p	-	-	Died of shock.
4	1	0.25 " " i/c 1.0 " " i/p	14	18	Ocular & nasal discharges.
5	2	1.0 ml sp. ex rabbit 4 i/p 2.0 " " s/c	12	17	Ocular & nasal discharges.
6	2	0.25 " " i/c 1.0 " " i/p	10	12	ditto. ditto.
7	3	1.0 " " 6 i/p	10	12	ditto. ditto.
8	3	0.5 " " "	13	14	Diarrhoea. Ocular & nasal discharges.
		Lost at passage 27.			

NOTE: gl ... gland; sp ... spleen; br ... brain; PI ... post-inoculation; i/c ... intra-cerebral; i/s ... intra-glandular; i/p ... intra-peritoneal; s/c ... sub-cutaneous

Intracerebral inoculations were made through the small space between the bones of the skull to be found about 15 mm. behind the base of the ear, using a 1 ml. all-glass syringe with a shortened 20-gauge needle. Light ether anæsthesia was employed.

Challenges. These consisted of the intraperitoneal inoculation of 0.5 to 2 ml. of blood, spleen or mixtures of spleen and gland.

Results

Adaptation.

Adaptation was successfully accomplished on four separate occasions (Table I, series I-IV). Transmissions proceeded through ten, four, eleven and fifty-six passages respectively. The strain used (D) adapted itself readily to the rabbit and, apart from a few "misses" at the first passage, regular transmissions were not difficult to accomplish apart from the usual danger of intercurrent infection with coccidiosis and pasteurellosis. There were occasions, however, when the virus seemed to fade out, although no particular change of technique had been adopted, and each of the series came to an end for no very clear reason. Apart from this, 0.5 to 1 ml. of citrated blood, spleen or gland regularly caused infection and death within a well-defined period.

Like most viral adaptations to unusual hosts, the initial transmission presented the only real difficulty. In each case infected bovine gland or a mixture of gland and spleen were used. In Series I the intracerebral route was used; in Series II and III a combination of the intracerebral and intraperitoneal routes and also the intraglandular route; and in Series IV a combination of the intraperitoneal and subcutaneous routes was successful.

Incubation periods.

These varied from seven to seventy-three days, a similar picture to that seen in cattle. But after the first few passages the great majority of reactions occurred between eight and fifteen days after inoculation (Figs. I and II).

Materials successfully used.

Transmissions were accomplished with rabbit brain, citrated blood, spleen, liver, lung, peritoneal fluid, lymph gland and kidney. Using blood preserved in O.C.G. mixture (oxalate-carbol-glycerine), the disease appeared only after a prolonged incubation period of 73 days in one case (rabbit 4, Series I) and not at all in another (rabbit 11, Series I).

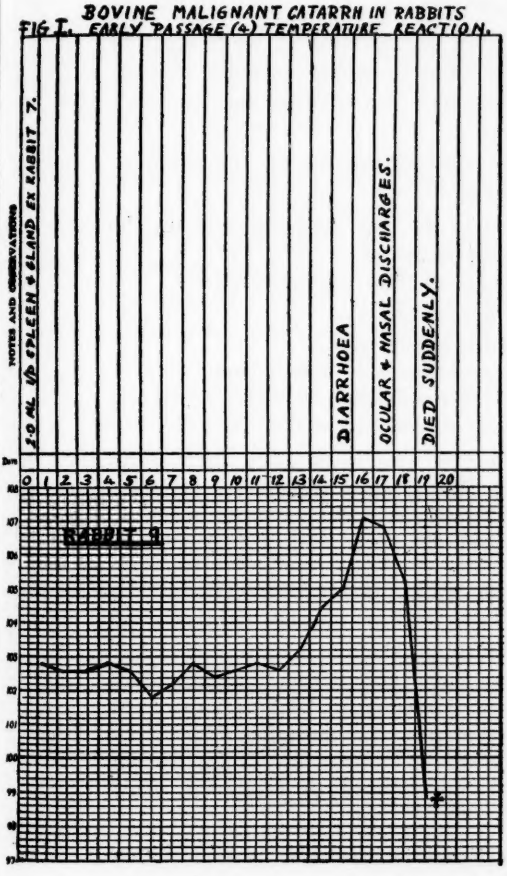
Routes of inoculation.

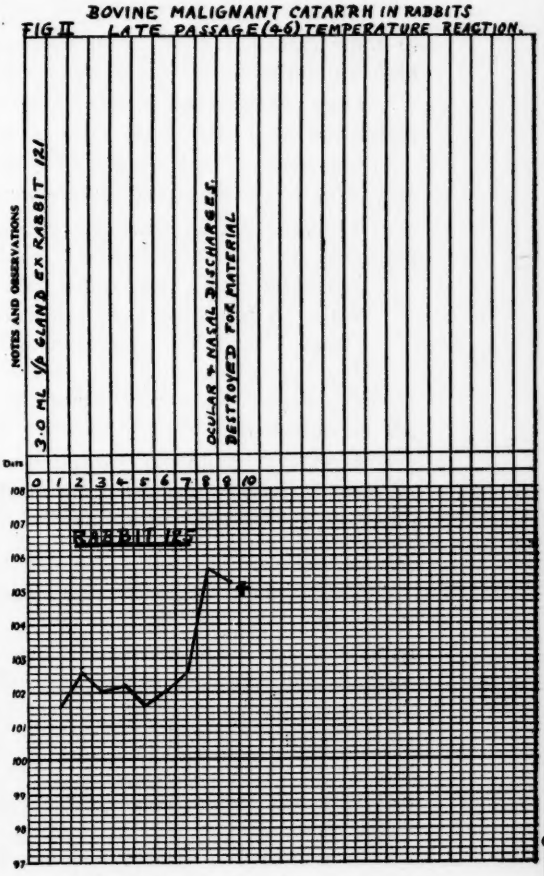
The intracerebral, intravenous, intraglandular, subcutaneous and intraperitoneal routes all were employed successfully.

Clinical symptoms and course.

Almost invariably the onset of symptoms was heralded by a sudden temperature rise of about 2° C. Usually temperatures continued to rise to a point between 106° and 107° C., occasionally higher, and then dropped suddenly prior to death. With well-adapted strains the course of recognisable disease was short, as a rule it was of two to three days' duration only. Little premonition of impending death was evident, the most reliable sign being a sudden and rapid fall in temperature, after which death occurred, in most cases, within a matter of hours.

Clinical symptoms varied with the route of inoculation and the stage of viral adaptation. Thus in early intracerebral passages various manifestations of brain damage were seen, such as the head twisted to one side, "circling," "paddling" of the limbs, muscular inco-ordination and partial or complete paralysis. In addition, ocular and nasal discharges becoming muco-purulent in the later stages and diarrhœa were seen. Using the intraperitoneal and intra-


BOVINE MALIGNANT CATARRH IN RABBITS
FIG I. EARLY PASSAGE (4) TEMPERATURE REACTION.
NOTES AND OBSERVATIONS
2·0 ML ½ SPLEEN + GLAND EX RABBIT 7.
DIARRHOEA
OCULAR + NASAL DISCHARGES.
DIED SUDDENLY.
Days
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
RABBIT 9


BOVINE MALIGNANT CATARRH IN RABBITS
FIG II LATE PASSAGE (46) TEMPERATURE REACTION.
NOTES AND OBSERVATIONS
3·0 ML ½ GLAND EX RABBIT 121
OCULAR + NASAL DISCHARGES.
DESTROYED FOR MATERIAL
Days
0 1 2 3 4 5 6 7 8 9 10
RABBIT 125

venous routes, symptoms were almost entirely confined to fever, anorexia, mild ocular and nasal discharges and some diarrhœa. In such cases the severity of symptoms tended to vary with the duration of the reaction. Often when the reaction was short, symptoms were almost entirely absent.

Post-mortem lesions.

Lymph glands. The lymph glands generally were enlarged, congested and often hæmorrhagic. Such changes were seen most frequently in the precrural, prescapular, inguinal and submaxillary lymph glands. The cut surface often

showed white granular patches, reminiscent of the disease in cattle, caused by necrosis and concentrations of large numbers of lymphocytic-type cells.

Spleen. Often this organ was enlarged and congested. Hypertrophy of the lymph nodes was not unusual. In some cases of prolonged reaction the spleen was enormous in size, dark liver in colour, the pulp being engorged and soft and showing irregularly distributed, large, rounded, paler areas.

Kidneys. Usually the kidneys appeared to be normal macroscopically, but in some cases leucocytic infiltration was seen in the form of white spots or blotches, similar to the typical kidney lesions found in bovine malignant catarrh.

Liver. Macroscopic changes were seldom seen, but friability and leucocytic infiltration were evident on some occasions.

Intestines. A mild degree of enteritis was not uncommon. Occasionally grey spots on the peritoneal surface were seen.

Appendix. A hæmorrhagic patch, 12 to 24 mm. long, midway on the peritoneal surface, was found on a number of occasions.

Peritoneal cavity. Sometimes an increase of fluid was observed.

Brain. A congested appearance was not uncommon.

Lungs. Frequently congestion and œdema were seen.

Trachea. Occasionally the mucous membrane was hæmorrhagic.

Turbinates. Some inflammation of the mucosa with a mucous catarrh was not uncommon.

Mortality. With the exception of one or two cases when material of doubtful infectivity was used, for example filtered brain (rabbit 12, Series I), infection was followed by death.

Histopathology.

This is not described in detail since the subject has been covered fully by Plowright (1953*a*, *b*). The most notable changes seen are the destruction of small lymphocytes in lymphatic tissue followed by the production in large numbers of medium and large lymphocytes, many of which are formed from undifferentiated cells, particularly around small blood vessels. Perivascular "cuffing," therefore, particularly in the brain, is common. Lymphocytic infiltration is followed by necrosis of smooth muscle and connective tissue in the spleen, lymph glands, meninges and other organs. Such changes are particularly striking in blood vessel walls.

It is of interest to note that inclusion bodies have never been seen, despite frequent and prolonged examinations.

Miscellaneous Experiments

Transmission to cattle.

Daubney and Hudson's (1936) demonstration that Koch's Postulates can be satisfied was repeated on three occasions. Using brain from rabbit 8 (Series I) at the fourth rabbit passage, cattle (813 and 953) were each given 5 ml. of material into the prescapular gland. One (813) reacted on the twentieth day, was destroyed *in extremis* six days later and classical lesions of B.M.C. demonstrated

at autopsy. The other (953) did not react and when challenged was found to be susceptible. On another occasion ox 1557 was given 10 ml. citrated blood from rabbit 39 (seventh passage, Series I) into the prescapular gland. It reacted and died of B.M.C. after a slightly extended incubation period of 27 days. A third attempt (oxen 3563 and 3108), using gland and spleen respectively from rabbit 113 (tenth passage, Series III), failed to initiate infection, although both steers were shown subsequently to have been susceptible. Finally, when spleen and gland from rabbit 21 (ninth passage, Series IV) were inoculated subcutaneously to cattle 4149 and 4120, both animals reacted and died of B.M.C., one after a very prolonged incubation period of nearly three months.

Titre of lapinised virus.

In a small experiment a 25 per cent suspension of brain from rabbit 6 (Series I) was diluted in tenfold series to a final concentration of 10^{-3}. Rabbits given undiluted material and the 1 : 10 dilution reacted and died, the latter after an extended incubation period of 44 days. Rabbits receiving the 1 : 100 and 1 : 1000 dilutions failed to react and were later shown to be susceptible. The indications are, therefore, that the titre of lapinised B.M.C. virus, as tested in rabbits, is of the same order as bovine virus titrated in cattle (Piercy, 1952).

Storage.

Although few experiments to determine the ability of lapinised B.M.C. virus to withstand storage were done, the results obtained confirm those pertaining to bovine virus. Thus, when rabbit citrated blood or brain suspension was stored at ordinary refrigerator temperatures for one or two days only, transmission was achieved. But when brain suspension was similarly stored for seven days, virus was not demonstrable by rabbit inoculation.

When spleen from rabbit 39 (Series I) was freeze-dried in ampoules, stored at —25° C., reconstituted after 12 days and given to rabbit 47 (Series I) no reaction occurred.

Adaptation to embryonated eggs.

On two occasions attempts were made to infect eggs with lapinised malignant catarrh virus. Eggs at the ninth day of incubation were inoculated by the chorio-allantoic route with infective rabbit brain. Post-inoculation incubation proceeded for four or five days at 95° F., when the membranes were harvested and subinoculated to a fresh series of eggs. Rabbits were inoculated with undiluted membrane from the third egg passage of one series and from the fifth egg passage of another. No reactions occurred in either case.

Experiments with antibiotics.

Both penicillin and streptomycin have been used regularly to control bacterial contamination and it needs no experiment to demonstrate that these two antibiotics fail to influence the course of the disease in rabbits.

When 0.037 g. chloromycetin was given daily, commencing from the day of inoculation, to two rabbits infected with lapinised malignant catarrh virus, reactions and death occurred in the usual periods of time.

Similar trials with aureomycin gave equally negative results.

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Adjuvant vaccine experiments.

Following the results of encouraging trials at the Kabete laboratories with adjuvant vaccines designed to protect cattle from rinderpest, a similar vaccine made from formalised infected rabbit spleen and gland and containing mineral oil, *Mycobacterium butyricum* and an emulsifying agent, was given to three rabbits in doses of 2 ml. subcutaneously. After a period of three weeks the rabbits were challenged and were shown to be susceptible.

Neutralisation with bovine serum.

Sera from oxen 3652 and 3607, both of which had resisted successive challenges with virulent bovine virus over periods of several months, were obtained. A group of six rabbits was inoculated as follows. Two rabbits received simultaneous inoculations of 2 ml. infective rabbit gland and spleen and 2 ml. of serum from ox 3652. Two rabbits received the same, except that serum from ox 3607 was used. Two rabbits received 2 ml. serum alone from oxen 3652 and 3607 respectively. The four rabbits given the virus/serum mixtures all reacted and died within the usual periods. The two serum control rabbits survived.

Discussion

The results reported in this article demonstrate clearly that rabbits can be infected with bovine malignant catarrh virus with relative ease and the virus maintained through successive generations, provided an adaptable strain is found. The latter point is important and several attempts with strains from different outbreaks may be necessary before successful adaptation is achieved. The usual difficulty in overcoming the resistance of foreign hosts during the first few generations must be expected (c.f. Pattison, 1946, and Series II of these articles) and it is advisable to use a generous number of rabbits for the initial transmission. In this connection there may be some value in inoculating rabbits simultaneously by the intracerebral and intraperitoneal routes, or by other combinations of routes.

With adequate care, and bearing in mind the vagaries of an extremely fragile virus propagated by artificial means, there seems to be no reason why transmission through the rabbit should not continue indefinitely. The intraperitoneal inoculation of 1 ml. citrated blood, spleen or gland, used fresh or stored not longer than 24 hours in the refrigerator, produced regular and uniform results in the series under discussion.

Several of the findings previously reported with bovine malignant catarrh (Piercy, *et al.*) have been confirmed in the rabbit experiments. For example, the failure of oxycarbol-glycerine mixture as a preservative; the rapid death of virus when stored as wet or dry material by recognised procedures, the failure to transmit the disease with filtered material, lack of success in attempts at propagation in fertile eggs, and the low titre of virus as judged by powers of infectivity.

There is some slight indication that attenuation for cattle may occur after a number of rabbit passages. Thus fourth passage rabbit material infected one out of two cattle; three passages later a steer reacted after an extended incubation

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period; ninth passage material killed two cattle, one of which reacted after a very extended incubation period; and tenth passage material failed to infect both animals inoculated. It seems doubtful, however, whether practical use could be made of an attenuated lapinised strain for immunisation purposes since relatively large amounts of fully-virulent material are required to infect cattle by artificial means and the immune response of such animals is poor (Piercy, 1954). No success was obtained with a formalised adjuvant vaccine.

The main value of adaptation to the rabbit would seem to be the opportunity presented for the evaluation of experimental therapy and prophylaxis and detailed pathological and histo-pathological studies, and possibly the elaboration of serological techniques.

Summary and Conclusions

1. The virus of bovine malignant catarrh has been adapted to rabbits on four separate occasions and passed through ten, four, eleven and fifty-six generations respectively. The importance of experimenting with various strains of virus, if necessary, for initial rabbit adaptation, is stressed.

2. A wide variety of rabbit tissues and routes of inoculation were employed successfully.

3. Clinical symptoms, the course of the disease, and post-mortem lesions in the rabbit are described.

4. Lapinised virus, returned to cattle at the fourth, seventh and ninth rabbit passages, caused death from malignant catarrh.

5. The characteristics of the lapinised virus as regards viability, infective titre, immunising ability and susceptibility to antibiotics remained similar to the parent laboratory strain " D."

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STUDIES ON HÆMORRHAGIC SEPTICÆMIA OF CATTLE

IV. A Preliminary Examination of the Antigens of *Pasteurella multocida* Type I

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Introduction

THE antigens of *Pasteurella multocida* type I which may be important in immunising have not been precisely characterised. Carter and Arnau (1953) have described the isolation of capsular polysaccharides from a Type B strain; Carter and Byrne (1953) have equated their type B with Roberts (1947) type I. Bain (1954 *a* and *b*) put forward the hypothesis based on the agglutinative and immunogenic behaviour of type I strains, that there may be a special antigen important in immunising. A rough analogy was drawn with the Vi antigen of the Enterobacteriaceæ. Bacteria able to immunise cattle effectively but relatively inagglutinable in immune serum were called "Phase I" as a convenient provisional term.

Materials and Methods

The "Insein" strain of *P. multocida* type I was used exclusively, as at present it is being employed in vaccination trials.

Extraction of Capsular substances. In all the methods tried the efficiency of extraction was controlled by microscopic checks. The aim was to extract as much capsular material as possible without disrupting the cells.

(a) *Direct Method.* The capsules of type I cells come into solution at 56° C. The process is helped by rotating mechanically the dense saline suspension in a flask with stainless steel ball bearings 2 mm. in diameter for two to four hours, the whole being held in a water bath at 56° C. This method was later discarded as it was feared, probably unjustifiably, that the antigens might be damaged at this temperature.

(b) *Amies' Method.* The work reported herein was done on fractions obtained by methods similar to those used by Amies (1951). To a dense suspension of bacteria in normal saline there was added an equal volume of molar potassium thiocyanate. The material was held in the incubator at 37° C. for five hours and then centrifuged. An opalescent straw-coloured supernatant was obtained. The cells were extracted for a second time overnight, recentrifuged, and the two supernatants were pooled. After centrifugation in the cold for two hours at 6,000 r.p.m., the fluid was passed through a sintered glass filter and then dialysed for 72 hours in the cold against numerous changes of distilled water. The dialysed fluid was then transferred to a beaker and the *pH* lowered by dropwise addition of N/1 HCl until clouding and then flocculation occurred. This took place at *pH* 3.5.

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The precipitate was removed by centrifugation and dissolved in phosphate buffered saline *pH* 7.0. A small portion usually remained insoluble. Reprecipitations decreased the solubility of the recovered fraction.

The supernatant was adjusted to *pH* 7.0, and half saturated with ammonium sulphate. This step was subsequently discarded as only a very small precipitate was recovered. The ammonium sulphate was removed by dialysis and the clear fluid was treated with ethanol plus a little sodium acetate. Excess acetate should be avoided as it co-precipitates with the biological material. Preliminary trials suggested that successive additions of 1, 1.5, 3 and finally 4 volumes of ethanol would remove fractions which might differ qualitatively, though it is not claimed that four different substances were obtained in this way. The fractions separated as fluffy precipitates and were recovered by centrifugation. The precipitates were readily soluble in water and were dialysed against distilled water before freeze drying. The material which separated with one volume of ethanol was white when dry but greenish in solution and contained a few insoluble flecks. The other fractions were white.

(c) *Saline extraction of acetone-killed cells* (Baker, Sommer, Foster, Meyer and Meyer, 1952). This method was used successfully on *P. pestis* by Baker *et al.* In view of the results obtained by Amies' method, a small batch of cells were processed. Fractions were obtained but not analysed.

Results

In the first experiment attempted, method (a) was used and the solution obtained was fractionated directly with ethanol on the assumption that the capsular antigens were likely to be polysaccharides. The fractions were tested for their capacity to immunise mice by injecting 1 mg. in two doses at intervals of ten days, followed by challenge with 100 $L.D._{50}$ ten days later. The results are shown in Table I.

TABLE I

Protective Power of Ethanol-precipitated Fractions for Mice

Fraction	Untreated	After shaking with $CHCl_3$
	Survivors	Survivors
1 vol. Ethanol	36/36	4/20
1.5 vol. "	12/36	1/20
3 vol. "	6/36	0/20
4 vol. "	5/36	0/20
Controls	0/10	0/10

Dose. 1.0 mgm. in two injections. Challenge 100 $L.D._{50}$

TABLE II

Properties of Iso-electrically removed Fraction

	P. multocida Type I	P. pestis (Amies, 1951)
Appearance	opalescent	opalescent
Nitrogen	11.5%	10%
Biuret	+	+
Millon	+	+
Hopine Cole	weak	weak
Sakaguchi	-	weak
Molisch	+	- or trace
Heating at 100°C	clouding	no visible change
Isoelectric pptn.	pH 3.5	pH 4.4

Both fractions tested at concentration of 5 mg/ml.

The fractions were then shaken mechanically with changes of chloroform and butanol until no more emulsion formed. Considerable emulsion formation occurred in the 1 volume ethanol fraction and little in the others. After being thus deproteinised the fractions had little ability to immunise mice in equivalent

dosage. (The dose was based on the same volume of antigen solution, less the material removed by the chloroform.)

The experience of Amies with *P. pestis* was similar to this, and his method of using thiocyanate to dissolve protein capsules was tried. Previously the method had been tried by the author on other *P. multocida* serotypes, without success. On the type I strain (Insein), however, it worked very well, though more slowly than Amies found it to work on *P. pestis*.

The properties of the isoelectric precipitate are set out in Table II, and Amies' results on the equivalent fraction from *P. pestis* are shown for comparison.

It has not been possible as yet to examine the physical properties of the fraction. Reprecipitations at the isoelectric point lead to progressive loss of solubility. The nitrogen value suggests considerable admixture with carbo-

TABLE III

Protective Power for Mice of Iso-electric and Ethanol Fractions

Fraction	Survivors	Fraction	Survivors
Isoelectric	14/20*	3 vol. ethanol	0/20
1 vol. ethanol	0/20	4 vol. ethanol	0/20
1.5 vol. "	1/20	Controls	0/9

Dose: 5.5 micrograms antigen nitrogen in 2 injections.
Challenge: 10 $L.D._{50}$.
* Some other preparations of 1 vol. ethanol fractions have protected up to 70% of mice.

TABLE IV

Yields of Iso-electric Fraction and Polysaccharides per Gram of Freeze-Dried Extracted P. multocida *Type I*

Source	Isoelectric	Polysaccharide
Agar-grown "Phase I" ‡ (Fluorescent Phase)	56 mgm.	81 mgm.
Broth-grown "Phase I" ‡ (Fluorescent Phase)	53 mgm.	25 mgm.
Agar-grown "non-Phase I" + (Intermediate Phase)	56 mgm.	19 mgm.

‡ Average of two preparations. + single preparation only.

hydrate. This is supported by the positive Molisch test. The fraction is similar to the Fraction IA of *P. pestis* (Baker *et al.*, 1952). A sample of Fraction IA was obtained through the courtesy of Dr. Karl Meyer. It does not react serologically with antisera to whole type I pasteurellas or to the isoelectric fraction.

Experiments with the Isoelectric Fraction. This fraction was found to be remarkably effective at immunising mice. Doses of the order of 1 mg. injected intraperitoneally as a saline solution regularly immunised 100 per cent of mice. After several trials it was found that 40 μg, given in two doses at ten-day intervals, would protect on the average 7/10 mice against 10 $L.D._{50}$, challenge being made ten days after the last dose.

A comparative test between the isoelectric fraction and the ethanol fractions (presumptive polysaccharides) was carried out. As we were unable to complete chemical analyses at this time, the dosage of the various fractions was based on their nitrogen content. It was already known that approximately 4.5 μg N of the isoelectric fraction would protect mice. Accordingly, concentrations of the vaccines were based on this figure. Two doses were given and the mice were challenged on the twenty-first day with 10 $L.D._{50}$ intraperitoneally. The results are shown in Table III.

The isoelectric fraction has been obtained from bacterial cells grown directly from infected blood on agar and also in broth. It has also been obtained from the " non-Phase I " variant which appears in early subcultivation of " Insein " or other type I strains. This variant is probably the " I " phase; on tryptose agar

it is not fluorescent, though still encapsulated. The capsules are smaller than those of the " F " phase and do not stain so well with alcian blue.

The yield of isoelectric fraction and polysaccharide from the above sources is shown in Table IV. For ease of comparison they are given as the yield per gram of extracted bacterial substance.

Pooled rabbit antisera against complete " Phase I " *P. multocida* type I are kept in this laboratory for typing. A dose of 0.1 ml. of the serum subcutaneously protects 100 per cent of mice against 1,000 L.D.$_{50}$ of " Insein." Portions of this serum were absorbed with equal amounts of the three isoelectric fractions (1 mg./ml. in three additions, after which there was no further precipitation). Groups of six mice were injected with 0.3 ml. of absorbed serum and challenged with 1,000 L.D.$_{50}$ 24 hours later, the usual practice. The protective power was abolished in all three absorbed sera. This indicates that the isoelectric fractions from " Phase I " and " non-Phase I " cells are qualitatively similar.

There has been little opportunity as yet for testing the immunising capacity of the iso-electric fraction in cattle. Two water buffaloes in Thailand were injected intramuscularly with 2 mg. and 1 mg. respectively of the fraction in an oil adjuvant emulsion. When examined a month later the animal receiving the 2 mg. dose showed strong agglutination, mouse protective and complement fixation reactions indicative of immunity, whereas the animal receiving 1 mg. showed only slightly increased serological activity. Both buffaloes were tested for natural immunity before injection.

In vaccination experiments in domestic cattle to be reported later, complement-fixation activity occurred in the sera of cattle vaccinated with agar-grown bacteria (8 out of 10), but not often in those receiving the equivalent dose of broth-grown cells (3 out of 26). Various antigens were used in the test, at first whole bacteria and then the fractions. " Phase I " bacterial suspensions grown on agar are poor antigens in the C.F. test; " non-Phase I " cells from agar are good; either form grown in tryptose broth is good. The antigen suspensions need to be about density 2 on the Burroughs Wellcome (Brown's tube) scale; these are not anticomplementary. Of the fractions, only the isoelectric precipitate fixes complement. The necessary concentration is 200 μg/ml., which is 50 times greater than that required for Fraction I of *P. pestis* (Chen, Quan and Meyer, 1952). The fraction only reacts with sera which give complement fixation with whole bacteria, but the titres are consistently about half. In 72 cattle and buffaloes tested with whole bacterial antigen, complement fixation has only been found in those sera showing mouse-protective antibodies. However, animals with strongly agglutinating or mouse-protective sera commonly fail to give a positive C.F. test. This may be due to a deficiency in the techniques employed.

Toxicity. The crude isoelectric fraction is toxic for rabbits, but much less so for mice. Sixty mice were given doses of from 10 μg to 1 mg. intravenously and all survived. Those receiving 1 mg. looked sick for a few hours, but recovered. Among 26 rabbits receiving graded doses intravenously, deaths occurred so erratically that assessment of an L.D.$_{50}$ was not possible. Rabbits receiving doses

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as low as 50 μg died and some deaths and survivors occurred at all doses up to 1 mg. Some rabbits died within two hours, others overnight, irrespective of the dose received. This makes the production of antisera difficult in rabbits unless they are started at a low dose level. An oil adjuvant base would probably be helpful.

Two calves, one week of age, were given 7 mg. and 3 mg. respectively of isoelectric fraction solution intravenously. Three hours later both were found prostrated, but they recovered overnight. A "shock reaction" in cattle following the injection of H.S. bacterin is widely recognised in S.E. Asia. Commonly the animals die. The reaction may be due to a substance in the isoelectric fraction. It is not known at present whether this fraction has one or more components.

Polysaccharide Fractions. These have not been examined in detail. As set out briefly in Table I, the fractions brought down with 1.5, 3 or 4 volumes of ethanol and shaken with chloroform had little capacity to immunise mice in moderate dosage. This has been found for fractions prepared by methods (a) and (b). When method (b) was used the first ethanol fraction (1 volume) removed after isoelectric precipitation protected mice irregularly in various preparations. Although the nitrogen content of these did not exceed 2 per cent, it is possible that they contain variable contaminating amounts of the high-nitrogen material which comes down mainly in the isoelectric precipitation. A carbohydrate immunogenic for mice may be associated with the nitrogenous portion of the isoelectric fraction. The fraction is Molisch-positive. In several attempts by heating at 100° C. for 30 minutes, by treatment with papain or by trypsin, it has not proved possible to abolish the protective power for mice (40 microgram doses), though in every instance protection has been reduced by about 50 per cent.

The fractions precipitated with 3 or 4 volumes of ethanol contained an average of 1.5 per cent nitrogen, about 50 per cent hexosamine (Morgan and Elsons' method) but no uronic acid (Dische's method). The Kass and Seastone test for hyaluronic acid was negative. The absence of uronic acid and negative Kass and Seastone test suggest that hyaluronic acid is not present. Bull testis hyaluronidase has no effect on the capsules of *P. multocida* type I, though it promptly decapsulates types II, III and IV. Mucoid variants of type I have not been studied in this laboratory; Carter and Annau (1953) reported a positive Kass and Seastone test with a fraction from an M variant.

All the fractions gave positive precipitin tests at a dilution of 1 : 100,000 with rabbit antisera to whole bacteria.

Agar-grown cells gave the highest yield of poly-saccharide (Table IV). Care was taken to exclude agar from the harvested cells, but the possibility remains that some was absorbed to the cell surfaces.

Discussion

At this stage it appears that *P. multocida* type I is similar in its antigenic structure to *P. pestis*. The protein-complex which is removed by isoelectric

precipitation from a dialysed solution of capsular substance is akin to Amies' fraction and also to the Fraction IA of Baker *et al.* (1952) in its capacity to immunise and to act as a complement-fixing antigen. It is unlikely that it is a purely somatic antigen because antisera prepared against it agglutinate intact capsulated bacilli. Its part in immunising cattle is not clear at present. The semi-purified polysaccharides alone have little capacity to immunise mice. Their effect on cattle is unknown. The "Phase I" properties of *P. multocida* type I are attributable in part at least to their relatively high polysaccharide content compared to the variants which emerge after subcultivation. The yield of isoelectric fraction from " Phase I " and " non-Phase I " cells is similar.

The mechanism of immunity to hæmorrhagic septicæmia does not appear to have been studied. Attempts by the author to find bactericidal action in fresh immune sera have failed consistently. Phagocytosis occurs in whole blood from immune animals, but the indices obtained by the methods tried to date have been low. If bacteriotropins are important in immunity, polysaccharides on the intact cell may be important antigens.

The special capacity of agar-grown cells to stimulate formation of complement-fixing antibodies cannot be explained. The vaccines used in that experiment were standardised by opacity and were not analysed chemically. The average amount of dried bacterial substance in a dose of vaccine is 2.2 mg. The highest value obtained for isoelectric fraction from agar-grown cells is 7 per cent, and the lowest from broth cells 5.3 per cent. This discrepancy could be important at threshold levels if similar values held for the above vaccines.

The terms " Phase I " and " non-Phase I " are used with some reserve. Other terms are in use in the literature on pasteurellas such as " Mucoid," " Fluorescent," " Intermediate," " Blue," " Smooth," " Rough " (Carter and Annau, 1953; Elberg and Chen-Lee Ho, 1950; Anderson, Coombes and Mallick, 1929). Distinction is not always drawn between phenotypic and genotypic variation. On simple media " Phase I " *P. multocida* type I behaves as if it produced less polysaccharide by being more agglutinable in antisera, less agglutinable in cetyl trimethyl ammonium bromide, and less stainable by alcian blue on the surface. On transfer to richer media, especially if much carbohydrate is present, it behaves as if more polysaccharide were being synthesised. This kind of variation is environmental. The " non-Phase I " bacilli are true genetic variants which do not revert to " Phase I " on culture. They appear in cultures initially along with " Phase I " (F) colonies. Both types can be maintained as pure lines from the parent culture. When cultures are transferred blindly without colony selection, the " non-Phase I " variants become progressively more dominant. Table IV shows that the main loss is in polysaccharide; the intact cells from which this " non-Phase I " preparation was made have failed to produce any evidence of immunity in two calves in adequate dosage. It remains to be seen whether this is due to lack of polysaccharide, or a qualitative difference in the isoelectric fraction. Another possible explanation is that the ease with which fractions are extracted may vary and that " Phase I " cells contain more

isoelectric fraction than the analysis shows. This could escape the visual check which is made for efficiency of extraction. The extracted cell residues from all three preparations in Table IV protected mice equally well in doses of 40 μg, the same dose which was used for the extracted isoelectric fraction.

Summary

A protein-complex fraction has been isolated from the capsules of *P. multocida* type I along with variable quantities of polysaccharide. The protein antigen is grossly similar to fractions extracted from *P. pestis*. For mice it is an important antigen conferring active immunity in small doses and removing the protective power from rabbit antisera to whole bacilli. It acts as a complement-fixing antigen with cattle sera. The yield from " Phase I " (F) and " non-Phase I " (Intermediate) is similar, but these show differences in the yield of polysaccharides.

Acknowledgment

The author is indebted to Mr. R. F. Jones of this Department for assistance with the fractionations and analyses.

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Footnote

The Author informs us in a subsequent communication that the two buffaloes receiving 2 mg. and 1 mg. respectively of isoelectric fraction were challenged with 1,000 L.D. four months later. Both were immune and both control buffaloes died.

DEATHS IN SWANS ASSOCIATED WITH TREMATODE INFECTION

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DURING the spring of 1954 the death of several mute swans (*Cygnus alor* [Gmelin]) occurred on the river Axe in Somerset. Initially, poisoning was suspected but post-mortem examination of four swans revealed a very heavy infestation of a trematode which was identified as *Echinoparyphium recurvatum* (V. Linstow, 1873).

Altogether eight swans died during 1954, six deaths occurring during March and April and two in September. The swans were found dead without

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any previous evidence of disease; however, as they were not tame it is unlikely that any signs of ill-health would be noted. All the deaths occurred within a radius of one mile and the majority were found in a long "cul-de-sac" part of the river. This part of the river was current free, it contained an abundance of weed and large numbers of snails were present on the muddy banks. The swans were said to feed by skimming the surface of the mud and water with their beaks, thus inadvertently or otherwise picking up large numbers of snails.

Post-mortem examination of four swans showed the following features: Each carcase was emaciated and the vent grossly contaminated with diarrhœic fæces. Internally there was a marked absence of fat. The small intestine, particularly the duodenum, showed a marked catarrhal enteritis and large numbers of small whitish trematodes were visible on the mucosa. A more detailed examination of the duodenal contents showed adult and immature trematodes, the proportion being of the order of one adult to two immature stages.

The identification as *E. recurvatum* was based on the presence of the general characters of the family *Echinostomatidæ*, a complete collar bearing two rows of spines differing in size in the two rows (Genus *Echinoparyphium*), the presence of 45 spines on the collar, four on each side being corner spines and the smallest size of spines being not less than 0.049 mm. (species *E. recurvatum*).

The parts of the river where deaths and feeding occurred were visited and a large number of various species of snails was collected, including snails of the genera *Limnea, Planorbis, Valvata* and *Vivipara.* These were examined for the characteristic cercariæ with a double circlet of collar spines but none of the snails were infected.

Discussion

The presence of emaciation and diarrhœa accompanied by large numbers of trematodes indicates that the deaths were closely associated with the parasitic infection. The presence of many immature flukes would suggest that the infestation was acute, the swans having ingested large numbers of cercariæ in a short period.

The intermediate hosts of *E. recurvatum* are given as *Valvata piscinalis* (Harper, 1929) and *Paludina vivipara* (Dinulisco, 1939); encysted cercariæ may also occur in *Planorbis albus* (Harper, loc. cit.). Since the deaths occurred at a particular part of the river where these intermediate hosts were likely to occur in large numbers and at time when snail populations were increasing, it would indicate that these particular conditions are necessary before a high mortality rate can occur. Brumpt and Buttner (1949) have stated that tadpoles of *Rana temporaria* may act as intermediate hosts for *E. recurvatum,* the cercariæ encysting in the kidneys of tadpoles. The part of the river in question was said to be a rich source of tadpoles and these may have contributed to the severity of the swan infestation.

As far as can be ascertained this is the first record of *E. recurvatum* in the mute swan. Brauer (1909) gives the hosts as the Scaup duck (*Nyroca marila marila*), Tufted duck (*N. fuligula*) and hen (*Gallus domesticus*); Dawes (1946)

extends the host list to include the domestic duck (*Anas*), pigeon (*Columba palumbus palumbus*), goosander (*Mergus merganser merganser*) and pintail (*Dafila acuta acuta*). Neveu-Lemaire (1936) includes also "several species of wild duck."

Annereaux (1940) has recorded the infestation in a turkey, the lesion being a severe inflammation of the intestinal mucosa. Lesions produced by a closely related parasite, *E. paraulum*, in chickens are stated to be a hæmorrhagic enteritis (Amundsen and Økland, 1936). It is apparent, therefore, that the type of lesion in the mute swan is not uncharacteristic of that to be expected in *Echinoparyphium* infestation.

Summary

E. recurvatum is recorded in the mute swan and epidemiological considerations are discussed.

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REVIEW

The Practice of Veterinary Medicine, by D. H. Udall, Prof. of Veterinary Medicine, Cornell University. London: Baillière, Tindall & Cox, Ltd. Price 65s.

The sixth edition of this book will enhance the reputation already established by the previous five editions which have been published since 1933. Its value and appeal is world wide, as a glance at the references given at the end of each disease section will prove. The emphasis is naturally on the disease picture amongst the farm animals of the author's own country and experience, but the majority of the conditions mentioned occur elsewhere than in the U.S.A. and the geographical incidence and relative importance as usually indicated.

As the author mentions in his preface, it is difficult to know what to omit when revising a veterinary medicine textbook and consequently the amount of material contained in the fifth but omitted in the sixth edition has been kept to a minimum. Additional monographs on the disease pictures which have become established since the publication of the fifth edition have necessitated the expansion of the book by more than 200 pages. These new monographs include such conditions of fairly recent interest in this country as atrophic

rhinitis, leptospirosis, molybdenum excess with copper deficiency, and insecticide poisoning. Amongst various revisions the chapter on mastitis has been considerably and usefully expanded.

In revising the older monographs, Professor Udall has modernised the therapeutic parts of the disease sections so that more recent treatments with the newer specific drugs such as terramycin and aureomycin are included. It is obvious, with such a monumental task, that some details will be overlooked. In this category are such surprising recommendations as the use of "alcoholic sublimate 1 : 500" as a personal antiseptic and "for immediate relief of low choke in the horse" the use of water injection and back siphoning through a stomach tube.

In choosing the new material one would have liked to read the author's comments on such conditions as trichomoniasis, vibrionic abortion and leukorrhœa of cattle, ergotism, and perhaps ringworm in horses and pigs.

These inclusions and exclusions, however, in no way detract from the exceedingly high standard of presentation and the integrity of the information.

The standard of proof reading has also been exceedingly high for there are very few errors, either in the "printer's error" category or of a grammatical and sense nature. The printing and binding are strictly in the tradition of the publishers who must be congratulated along with Professor Udall and his colleagues for producing this new addition to a distinguished line.

NOTICES

ALLEN & HANBURYS LTD. announce that to meet the needs of large users of their Veterinary Alficetyn Paint, a 500 c.c. size, with brush, has been introduced. They have also introduced a Canine Distemper Vaccine (Egg Adapted-Living). This is a freeze-dried product for the immunisation of dogs against distemper and hard-pad. Prices and further particulars may be obtained from the manufacturers, Bethnal Green, London, E.2.

BURROUGHS WELLCOME & Co. announce the issue of "Temadex" brand Skin Dressing (Veterinary) for the treatment of parasitic and other skin conditions in dogs. They state that the inclusion of three important acaricidal agents in balanced proportions, together with an antifungal substance, ensures an efficient and versatile skin dressing with a wide range of activity. "Temadex," which is available to the veterinary profession only, is issued in boxes of 6 x 2 oz. tubes (dispensing pack). They also introduce "Aerosporin" brand Compound Ointment (Veterinary) for the treatment of infected wounds, and infections or the ear and eye in small animal practice. The combination of the two antibiotics, polymyxin B and bacitracin in this ointment provides antibacterial action which covers a wide range of organisms, both gram-positive

and gram-negative. The manufacturers claim that the inclusion of benzocaine greatly enhances its use in the treatment of painful conditions of the eye and ear. "Aerosporin" Compound Ointment is issued in tubes of 10 grammes with special nozzle.

Another product by the same firm is "Wellcome" brand Penicillin and Dihydrostreptomycin Intramammary Injection. This product, containing 300,000 units Procaine Penicillin G and 250,000 units Dihydrostreptomycin Sulphate in a bland base, covers a wide range of bacterial pathogens in bovine mastitis and is therefore particularly suited for the treatment of cases of mixed infection, and in such of unknown ætiology. The high concentrations in which both antibiotics are presented ensure the maintenance of adequate therapeutic levels in the udder. "Wellcome" Penicillin and Dihydrostreptomycin Intramammary Injection is issued in single-dose tubes, in boxes of 12.

Benger Laboratories Ltd. announce the introduction of a new product, "Imferon" Veterinary, for the single dose control of piglet anæmia. "Imferon" was first developed for the treatment of human anæmia and its efficiency for that purpose is now fully established. A veterinary version of the product has just become available (to the veterinary profession only) and was shown for the first time at the British Veterinary Association Congress at Belfast. The product is given by intramuscular injection and it is claimed that a single dose will ensure survival of the piglet and produce a normal hæmoglobin concentration by the time the animal is three to four weeks old. "Imferon" Veterinary is presented in multidose containers of 20 ml. and is distributed by: Boots Pure Drug Co. Ltd., Nottingham; C. J. Hewlett & Son Ltd., Watford; Willington Medicals Ltd., Shrewsbury; Willows Francis (Pharmaceutical Products) Ltd., London.

Evans Medical Supplies Ltd. have introduced Nemazine, an anthelminthe for veterinary use. This is a preparation of Phenothiazine dispersible powder with copper and cobalt added. It is supplied in 1 lb. and 7 lb. tins with 5g. measuring scoop.

This firm has also introduced a special veterinary pack of their product Fotogel. This is a Barium Sulphate suspension for X-ray diagnosis and the veterinary container has a strengthened delivery nozzle to withstand bites of small animals and assist administration.

Burroughs Wellcome & Co. announce that the title of "Wellcome" brand Protein Hydrolysate has now been changed to "PROTOGEST" brand Protein Hydrolysate. The product continues to be issued in packs of 6 x 100 c.c. bottles. They also announce that the title of "Wellcome" brand Sodium Propionate Compound for the treatment of Bovine Acetonæmia is now changed to "MELINAX" brand Sodium Propionate Compound. The pack is a tin of 30 oz.

ITEMS OF VETERINARY INTEREST

THE Minister of Agriculture, Fisheries and Food and the Secretary of State for Scotland, after consultation with the Agricultural Research Council and the Medical Research Council, have jointly appointed Mr. Stephen Cheveley, O.B.E., to be Chairman of the Governing Body of the Foot-and-Mouth Disease Research Institute at Pirbright, Surrey, in succession to Professor Wilson Smith, M.D., F.R.S., who is retiring from the Governing Body.

Mr. Cheveley's appointment as Chairman takes effect from October 1, 1955.

The Ministers wish to take this opportunity of expressing their appreciation of Professor Wilson Smith's valuable services to the development of research into foot-and-mouth disease.

THE Second World Congress on Fertility and Infertility is to take place in Naples from May 18 to 26, 1956. Section 12 deals entirely with problems in animal reproduction. Those wishing to take part should communicate at once with Professor T. Bonadonna, Associazione Internazionale della Fertilita, via Monte Ortigara, 35, Milan, Italy.

The Animal Health Trust have issued a pamphlet dealing with humane destruction of surplus chicks. This is available free on request from the Trust's headquarters, 14 Ashley Place, Westminster, London, S.W.1. It has been issued in collaboration with the Chick Producers' Association after consultation with the Royal Society for Prevention of Cruelty to Animals.

The Ministry of Agriculture, Fisheries and Food state that if the present rate of progress in eradication of tuberculosis from cattle continues it is expected that Great Britain will be free of the disease inside ten years.

The November issue of " Veterinary Medicine " is a Golden Jubilee number representing 50 years of continuous publication. This journal is among the leading veterinary periodicals in the United States and we take the opportunity to congratulate the publishers on their fine record of service to the veterinary profession.

WE have received from Messrs. Evans Medical Supplies Ltd. a copy of a brochure which was published at the time of the Veterinary Congress in Belfast. It contains a number of cave paintings which have been reproduced in advertisements for veterinary products by this firm. The interest aroused was such that it was thought that many would like to have them assembled under one cover.

A copy will be gladly sent to any Veterinary Surgeon or Veterinary Student on request.

XUM

THE NEW PFIZER ANTIBIOTICS FERMENTATION PLANT

THE new Pfizer antibiotics fermentation plant was opened in Sandwich on September 30. The plant has been built to supply the sterling area with terramycin which, until recently, had to be imported from America for processing into various clinical and agricultural forms.

John J. Powers, Jnr., Senior Vice-President of the American company, came from New York for the ceremony and introduced Lord Brabazon of Tara to an audience of distinguished guests. Among them was Lady Fleming, widow of Sir Alexander Fleming, the President of the Royal College of Veterinary Surgeons, Mr. H. Sumner, Mr. J. L. McGirr representing Dr. A. W. Stableforth, Director of Veterinary Research for the Ministry of Agriculture. Many heads of scientific departments, colleges and universities were among the guests, and Mr. John J. Powers said :—

"We are gathered here at the commencement of what we hope is the most modern plant of its kind in Europe. It is a British plant, conceived by British brains, installed by British hands, and operated by Britishers. I speak to you to-day as an American because it is an opportunity to put into words a sense of appreciation for the beginning here in this land by a wonderful man of a great era.

"I am referring, of course, to the discovery of penicillin by our dear friend Sir Alexander Fleming, and all that followed. I think that I may presume to speak for the scientific world of America in saying to-day that this is only a small return to English soil of what, one day before, we took from it.

" These times move fast with respect to antibiotics considering the fact that the wonderful drug, penicillin, was first brought to our use only a decade ago. It has a range of effectiveness against about 25 pathogenic micro-organisms which cause disease, but by contrast, the drug terramycin, which is one of those which will be manufactured in this plant, has an effectiveness against about 100 pathogenic micro-organisms. Consider the wide range of disease which has been halted.

" I think I might add that terramycin is not just a contribution to the medical world. In over 2,000 papers which have been published by investigators of this drug, many are devoted not only to its medical advantages but to the fact that another new era is open—that of animal nutrition represented by feed supplements made available to the farmers throughout the world."

Mr. J. A. Rodgers concluded the official opening by reminding the gathering that terramycin would no longer cost dollars but would become a British export to doctors and veterinary surgeons in the sterling area. The guests then dispersed to tour the fermentation plant.

Publishers' Notices

THE BRITISH VETERINARY JOURNAL, with which is incorporated THE VETERINARY JOURNAL, is published monthly, and copy for advertisements should be in the hands of the advertisement manager not later than the 20th of the preceding month, if proof is required. Tel.: Temple Bar 3386.

Letters for the Journal, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

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